```
  1 TTTCCTCACTGACTATAAAAGAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC

  1 ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGT
  1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys

181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAA
 31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu

271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
 61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys

361 ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCNCAG
 91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln
                                                            *
451 AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA
121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys

541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGG
151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly

631 TTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATT
181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle

721 TACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr

811 TCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCAT
241 SerIleTyrGlnGlyGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis

901 GAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGAT
271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp

991 GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAA
```

FIG._1

BEST AVAILABLE COPY

```
  1 ATGGCGCCAC CACCAGCTAG AGTACATCTA GGTGCGTTCC TGGCAGTGAC
    TACCGCGGTG GTGGTCGATC TCATGTAGAT CCACGCAAGG ACCGTCACTG
  1 MetAlaProP roProAlaAr gValHisLeu GlyAlaPheL euAlaValTh

 51 TCCGAATCCC GGGAGCGCAG CGAGTGGGAC AGAGGCAGCC GCGGCCACAC
    AGGCTTAGGG CCCTCGCGTC GCTCACCCTG TCTCCGTCGG CGCCGGTGTG
    rProAsnPro GlySerAlaA laSerGlyTh rGluAlaAla AlaAlaThrPro

101 CCAGCAAAGT GTGGGGCTCT TCCGCGGGGA GGATTGAACC ACGAGGCGGG
    GGTCGTTTCA CACCCCGAGA AGGCGCCCCT CCTAACTTGG TGCTCCGCCC
 35   SerLysVa lTrpGlySer SerAlaGlyA rgIleGluPr oArgGlyGly

151 GGCCGAGGAG CGCTCCCTAC CTCCATGGGA CAGCACGGAC CCAGTGCCCG
    CCGGCTCCTC GCGAGGGATG GAGGTACCCT GTCGTGCCTG GGTCACGGGC
    GlyArgGlyA laLeuProTh rSerMetGly GlnHisGlyP roSerAlaArg

201 GGCCCGGGCA GGGCGCGCCC CAGGACCCAG GCCGGCGCGG GAAGCCAGCC
    CCGGGCCCGT CCCGCGCGGG GTCCTGGGTC CGGCCGCGCC CTTCGGTCGG
 68  AlaArgAla GlyArgAlaP roGlyProAr gProAlaArg GluAlaSerP

251 CTCGGCTCCG GGTCCACAAG ACCTTCAAGT TTGTCGTCGT CGGGGTCCTG
    GAGCCGAGGC CCAGGTGTTC TGGAAGTTCA AACAGCAGCA GCCCCAGGAC
    roArgLeuAr gValHisLys ThrPheLysP heValValVa lGlyValLeu

301 CTGCAGGTCG TACCTAGCTC AGCTGCAACC ATGATCAATC AATTGGCACA
    GACGTCCAGC ATGGATCGAG TCGACGTTGG TAGTTTGAAG TACTAGTTAG
101 LeuGlnValV alProSerSe rAlaAlaThr IleLysLeuH isAspGlnSe

351 AATTGGCACA CAGCAATGGG AACATAGCCC TTTGGGAGAG TTGTGTCCAC
    TTAACCGTGT GTCGTTACCC TTGTATCGGG AAACCCTCTC AACACAGGTG
    rIleGlyThr GlnGlnTrpG luHisSerPr oLeuGlyGlu LeuCysProPro

401 CAGGATCTCA TAGATCAGAA CGTCCTGGAG CCTGTAACCG GTGCACAGAG
    GTCCTAGAGT ATCTAGTCTT GCAGGACCTC GGACATTGGC CACGTGTCTC
135   GlySerHi sArgSerGlu ArgProGlyA laCysAsnAr gCysThrGlu

451 GGTGTGGGTT ACACCAATGC TTCCAACAAT TTGTTTGCTT GCCTCCCATG
    CCACACCCAA TGTGGTTACG AAGGTTGTTA AACAAACGAA CGGAGGGTAC
    GlyValGlyT yrThrAsnAl aSerAsnAsn LeuPheAlaC ysLeuProCys

501 TACAGCTTGT AAATCAGATG AAGAAGAGAG AAGTCCCTGC ACCACGACCA
    ATGTCGAACA TTTAGTCTAC TTCTTCTCTC TTCAGGGACG TGGTGCTGGT
168  ThrAlaCys LysSerAspG luGluGluAr gSerProCys ThrThrThrA

551 GGAACACAGC ATGTCAGTGC AAACCAGGAA CTTTCCGGAA TGACAATTCT
    CCTTGTGTCG TACAGTCACG TTTGGTCCTT GAAAGGCCTT ACTGTTAAGA
    rgAsnThrAl aCysGlnCys LysProGlyT hrPheArgAs nAspAsnSer

601 GCTGAGATGT GCCGGAAGTG CAGCACAGGG TGCCCCAGAG GGATGGTCAA
    CGACTCTACA CGGCCTTCAC GTCGTGTCCC ACGGGGTCTC CCTACCAGTT
201 AlaGluMetC ysArgLysCy sSerThrGly CysProArgG lyMetValLy

651 GGTCAAGGAT TGTACGCCCT GGAGTGACAT CGAGTGTGTC CACAAAGAAT
    CCAGTTCCTA ACATGCGGGA CCTCACTGTA GCTCACACAG GTGTTTCTTA
    sValLysAsp CysThrProT rpSerAspIl eGluCysVal HisLysGluSer
```

FIG. 2A

```
701 CAGGCAATGG ACATAATATA TGGGTGATTT TGGTTGTGAC TTTGGTTGTT
    GTCCGTTACC TGTATTATAT ACCCACTAAA ACCAACACTG AAACCAACAA
235   GlyAsnGl yHisAsnIle TrpValIleL euValValTh rLeuValVal

751 CCGTTGCTGT TGGTGGCTGT GCTGATTGTC TGTTGTTGCA TCGGCTCAGG
    GGCAACGACA ACCACCGACA CGACTAACAG ACAACAACGT AGCCGAGTCC
    ProLeuLeuL euValAlaVa lLeuIleVal CysCysCysI leGlySerGly

801 TTGTGGAGGG GACCCCAAGT GCATGGACAG GGTGTGTTTC TGGCGCTTGG
    AACACCTCCC CTGGGGTTCA CGTACCTGTC CCACACAAAG ACCGCGAACC
268  CysGlyGly AspProLysC ysMetAspAr gValCysPhe TrpArgLeuG

851 GTCTCCTACG AGGGCCTGGG GCTGAGGACA ATGCTCACAA CGAGATTCTG
    CAGAGGATGC TCCCGGACCC CGACTCCTGT TACGAGTGTT GCTCTAAGAC
    lyLeuLeuAr gGlyProGly AlaGluAspA snAlaHisAs nGluIleLeu

901 AGCAACGCAG ACTCGCTGTC CACTTTCGTC TCTGAGCAGC AAATGGAAAG
    TCGTTGCGTC TGAGCGACAG GTGAAAGCAG AGACTCGTCG TTTACCTTTC
301 SerAsnAlaA spSerLeuSe rThrPheVal SerGluGlnG lnMetGluSe

951 CCAGGAGCCG GCAGATTTGA CAGGTGTCAC TGTACAGTCC CCAGGGGAGG
    GGTCCTCGGC CGTCTAAACT GTCCACATGT ACATGTCAGG GGTCCCCTCC
    rGlnGluPro AlaAspLeuT hrGlyValTh rValGlnSer ProGlyGluAla

1001 CACAGTGTCT GCTGGGACCG GCAGAAGCTG AAGGGTCTCA GAGGAGGAGG
     GTGTCACAGA CGACCCTGGC CGTCTTCGAC TTCCCAGAGT CTCCTCCTCC
335    GlnCysLe uLeuGlyPro AlaGluAlaG luGlySerGl nArgArgArg

1051 CTGCTGGTTC CAGCAAATGG TGCTGACCCC ACTGAGACTC TGATGCTGTT
     GACGACCAAG GTCGTTTACC ACGACTGGGG TGACTCTGAG ACTACGACAA
     LeuLeuValP roAlaAsnGl yAlaAspPro ThrGluThrL euMetLeuPhe

1101 CTTTGACAAG TTTGCAAACA TCGTGCCCTT TGACTCCTGG GACCAGCTCA
     GAAACTGTTC AAACGTTTGT AGCACGGGAA ACTGAGGACC CTGGTCGAGT
368   PheAspLys PheAlaAsnI leValProPh eAspSerTrp AspGlnLeuM

1151 TGAGGCAGCT GGACCTCACG AAAAATGAGA TCGATGTGGT CAGAGCTGGT
     ACTCCGTCGA CCTGGAGTGC TTTTTACTCT AGCTACACCA GTCTCGACCA
     etArgGlnLe uAspLeuThr LysAsnGluI leAspValVa lArgAlaGly

1201 ACAGCAGGCC CAGGGGATGC CTTGTATGCA ATGCTGATGA AATGGGTCAA
     TGTCGTCCGG GTCCCCTACG GAACATACGT TACGACTACT TTACCCAGTT
401  ThrAlaGlyP roGlyAspAl aLeuTyrAla MetLeuMetL ysTrpValAs

1251 CAAAACTGGA CGGAACGCCT CGATCCACAC CCTGCTGGAT GCCTTGGAGA
     GTTTTGACCT GCCTTGCGGA GCTAGGTGTG GGACGACCTA CGGAACCTCT
     nLysThrGly ArgAsnAlaS erIleHisTh rLeuLeuAsp AlaLeuGluArg

1301 GGATGGAAGA GAGACATGCA AAAGAGAAGA TTCAGGACCT CTTGGTGGAC
     CCTACCTTCT CTCTGTACGT TTTCTCTTCT AAGTCCTGGA GAACCACCTG
435    MetGluGl uArgHisAla LysGluLysI leGlnAspLe uLeuValAsp

1351 TCTGGAAAGT TCATCTACTT AGAAGATGGC ACAGGCTCTG CCGTGTCCTT
     AGACCTTTCA AGTAGATGAA TCTTCTACCG TGTCCGAGAC GGCACAGGAA
     SerGlyLysP heIleTyrLe uGluAspGly ThrGlySerA laValSerLeu

1401 GGAGTGA
     CCTCACT
468  GluOP*
```

FIG._2B

1 MEQRGQNAPAASGARKRHGPGPREARGARPGLRVPKTLVLVVAAVLLLVSAESALITQQD
61 LAPQQRAAPQQKRSSPSEGLCPPGHHISEDGRDCISCKYGQDYSTHWNDLLFCLRCTRCD
121 SGEVELSPCTTTRNTVCQCEEGTFREEDSPEMCRKCRTGCPRGMVKVGDCTPWSDIECVH
181 KESGIIIGVTVAAVVLIVAVFVCKSLLWKKVLPYLKGICSGGGGDPERVDRSSQRPGAED
241 NVLNEIVSILQPTQVPEQEMEVQEPAEPTGVNMLSPGESEHLLEPAEAERSQRRRLLVPA
301 NEGDPTETLRQCFDDFADLVPFDSWEPLMRKLGLMDNEIKVAKAEAAGHRDTLYTMLIKW
361 VNKTGRDASVHTLLDALETLGERLAKQKIEDHLLSSGKFMYLEGNADSALS

FIG._3A

Met	Glu	Gln	Arg	Gly	Gln	Asn	Ala	Pro	Ala	Ala	Ser	Gly	Ala	Arg	Lys
1				5					10					15	
Arg	His	Gly	Pro	Gly	Pro	Arg	Glu	Ala	Arg	Gly	Ala	Arg	Pro	Gly	Pro
			20					25					30		
Arg	Val	Pro	Lys	Thr	Leu	Val	Leu	Val	Val	Ala	Ala	Val	Leu	Leu	Leu
		35					40					45			
Val	Ser	Ala	Glu	Ser	Ala	Leu	Ile	Thr	Gln	Gln	Asp	Leu	Ala	Pro	Gln
	50					55					60				
Gln	Arg	Ala	Ala	Pro	Gln	Gln	Lys	Arg	Ser	Ser	Pro	Ser	Glu	Gly	Leu
65					70					75					80
Cys	Pro	Pro	Gly	His	His	Ile	Ser	Glu	Asp	Gly	Arg	Asp	Cys	Ile	Ser
				85					90					95	
Cys	Lys	Tyr	Gly	Gln	Asp	Tyr	Ser	Thr	His	Trp	Asn	Asp	Leu	Leu	Phe
			100					105					110		
Cys	Leu	Arg	Cys	Thr	Arg	Cys	Asp	Ser	Gly	Glu	Val	Glu	Leu	Ser	Pro
		115					120					125			
Cys	Thr	Thr	Thr	Arg	Asn	Thr	Val	Cys	Gln	Cys	Glu	Glu	Gly	Thr	Phe
	130					135					140				
Arg	Glu	Glu	Asp	Ser	Pro	Glu	Met	Cys	Arg	Lys	Cys	Arg	Thr	Gly	Cys
145					150					155					160
Pro	Arg	Gly	Met	Val	Lys	Val	Gly	Asp	Cys	Thr	Pro	Trp	Ser	Asp	Ile
				165					170					175	
Glu	Cys	Val	His	Lys	Glu	Ser	Gly	Thr	Lys	His	Ser	Gly	Glu	Ala	Pro
			180					185					190		
Ala	Val	Glu	Glu	Thr	Val	Thr	Ser	Ser	Pro	Gly	Thr	Pro	Ala	Ser	Pro
		195					200					205			
Cys	Ser	Leu	Ser	Gly	Ile	Ile	Ile	Gly	Val	Thr	Val	Ala	Ala	Val	Val
	210					215					220				
Leu	Ile	Val	Ala	Val	Phe	Val	Cys	Lys	Ser	Leu	Leu	Trp	Lys	Lys	Val
225					230					235					240
Leu	Pro	Tyr	Leu	Lys	Gly	Ile	Cys	Ser	Gly	Gly	Gly	Gly	Asp	Pro	Glu
				245					250					255	
Arg	Val	Asp	Arg	Ser	Ser	Gln	Arg	Pro	Gly	Ala	Glu	Asp	Asn	Val	Leu
			260					265					270		
Asn	Glu	Ile	Val	Ser	Ile	Leu	Gln	Pro	Thr	Gln	Val	Pro	Glu	Gln	Glu
		275					280					285			

FIG._3B

Met	Glu	Val	Gln	Glu	Pro	Ala	Glu	Pro	Thr	Gly	Val	Asn	Met	Leu	Ser
	290					295					300				
Pro	Gly	Glu	Ser	Glu	His	Leu	Leu	Glu	Pro	Ala	Glu	Ala	Glu	Arg	Ser
305					310					315					320
Gln	Arg	Arg	Arg	Leu	Leu	Val	Pro	Ala	Asn	Glu	Gly	Asp	Pro	Thr	Glu
				325					330					335	
Thr	Leu	Arg	Gln	Cys	Phe	Asp	Asp	Phe	Ala	Asp	Leu	Val	Pro	Phe	Asp
			340					345					350		
Ser	Trp	Glu	Pro	Leu	Met	Arg	Lys	Leu	Gly	Leu	Met	Asp	Asn	Glu	Ile
		355					360					365			
Lys	Val	Ala	Lys	Ala	Glu	Ala	Ala	Gly	His	Arg	Asp	Thr	Leu	Tyr	Thr
	370					375					380				
Met	Leu	Ile	Lys	Trp	Val	Asn	Lys	Thr	Gly	Arg	Asp	Ala	Ser	Val	His
385					390					395					400
Thr	Leu	Leu	Asp	Ala	Leu	Glu	Thr	Leu	Gly	Glu	Arg	Leu	Ala	Lys	Gln
				405					410					415	
Lys	Ile	Glu	Asp	His	Leu	Leu	Ser	Ser	Gly	Lys	Phe	Met	Tyr	Leu	Glu
			420					425					430		
Gly	Asn	Ala	Asp	Ser	Ala	Met	Ser	*							
		435					440								

FIG._3C

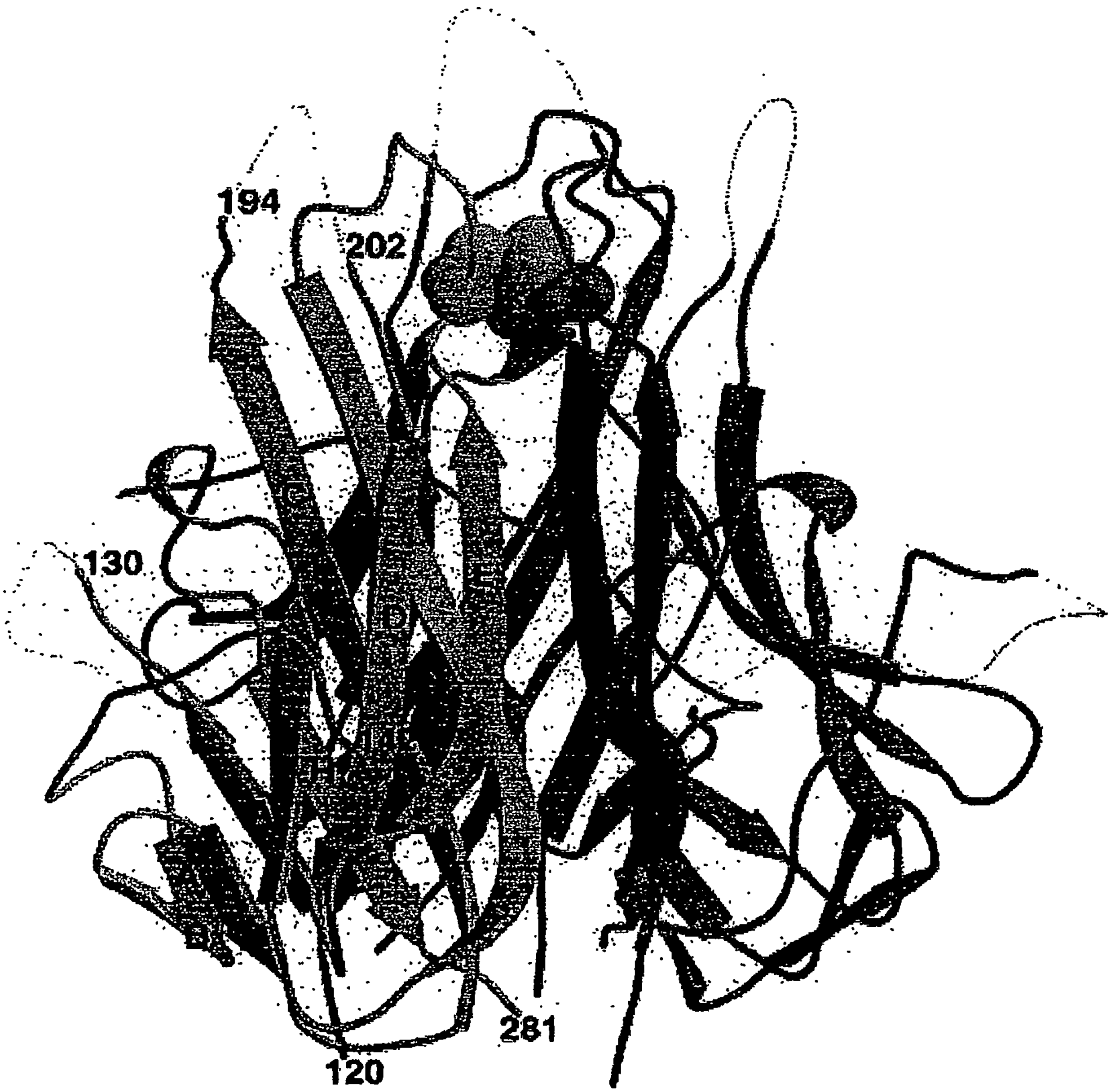


FIG._4A

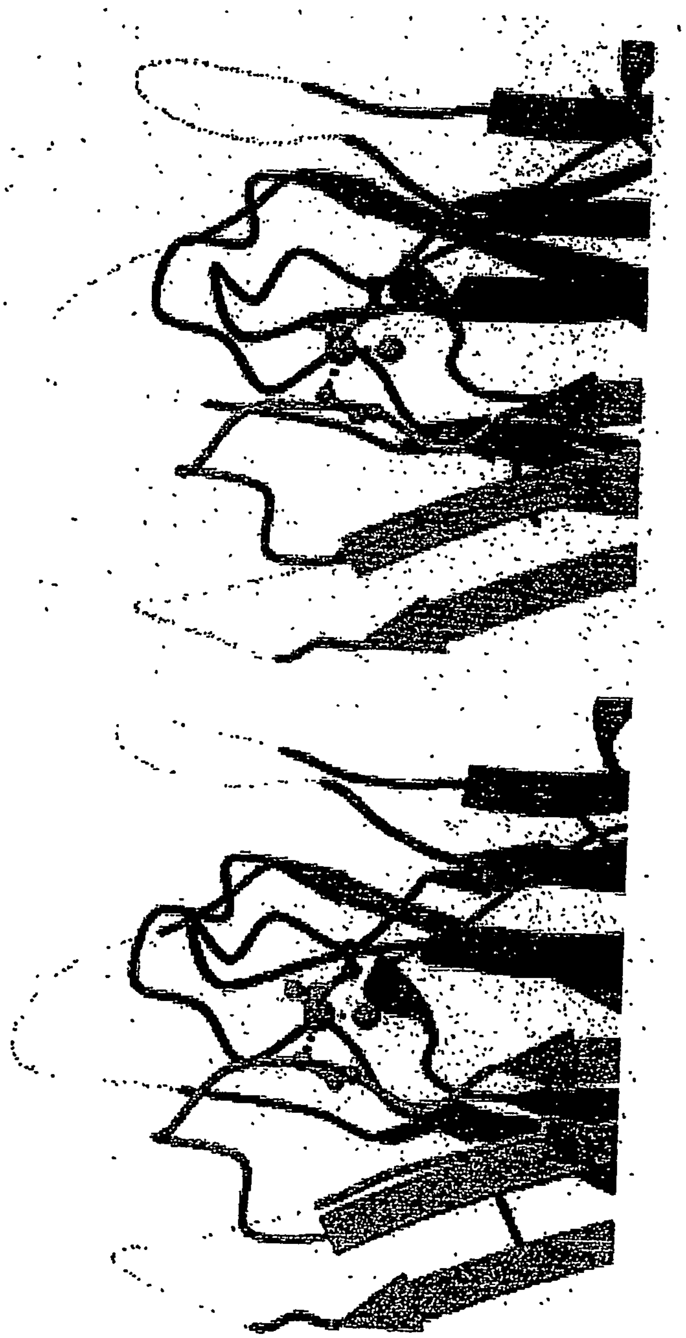


FIG._4B

Crystallographic Data

	Apo-2L (114-281)	Apo-2L (91-281) D218A	Apo-2L (91-281) D218A
Crystal			
Space Group	$P6_3$	R32	R32
Unit Cell (Å)	a=72.5 c=140	a=66.4 c=197.6	a=66.4 c=197.7
Resolution (Å)	3.9	1.9	1.3
Coverage (%)	94 (96)	93 (99)	100 (100)
$\langle I/\sigma(I)\rangle$	5.9	10.1	12.4
# Unique (hkl)	3589	12680	41840
Redundancy	4.9	4.3	12.1
R_{symm} (%)	15.4 (34)	6.2 (27)	6.4 (34)
# Protomers in ASU	2	1	1
Refinement			
R_{cryst} (%)	33.8	20	
R_{free} (%)	27.6	22	
rmsd Bonds (Å)	0.009	0.015	0.007
rmsd Angles (°)	1.79	2.0	1.41
Average B-Values	—	14	14
# Water Molecules	0	170	

Rsymm $=\Sigma_h\Sigma_i(I_{hi}-\langle I_h\rangle)/\Sigma_h I$ where I_h is the mean structure factor intensity of i observations of symmetry-related reflections with Bragg index h. $R_{cryst}=\Sigma_h\Sigma_i||F_{obs}|-|F_{calc}||)/\Sigma|F_{obs}|)$ where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes. $R_{free}=\Sigma_{(hkl)\varepsilon\tau}||Fobs_{(hkl)}|-k|F_{(hkl)}|/\Sigma_{(hkl)\varepsilon\tau}|Fobs_{(hkl)}|$ where the τ set of reflections is omitted from the refinement process. 10% of the data were included in the τ set for calculation of R_{free} and not included in refinement. Values in parenthesis are for the highest resolution shell.

FIG._4C

```
           A                                               A'   B'
 121      130        140        150        160       170
Apo2L  RVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLR
TNF-β  KPAAHLIGD................PSKQNSLLWRANTDRAFLQDGFSLS
TNF-α  KPVAHVVAN................PQAEGQLQWLNRRANALLANGVELR
CD40L  QIAAHVISE..............ASSKTTSVLQWAEKGYYTMSNNLVTLE
FasL   RKVAHLTGK...............SNSRSMPLEWEDTY.GIVLLSGVKYK
RANKL  QPFAHLTIN.........ATDIPSGSHKVSLSSWYHDR.GWAKISNMTFS

           B        C                  D
         180        190        200        210
Apo2L  NG.ELVIHEKGFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTS.YPD
TNF-β  NN.SLLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAHEVQLFSSQYPF
TNF-α  DN.QLVVPSEGLYLIYSQVLFKGQG....CPSTHVLLTHTISRIAVSYQT
CD40L  NGKQLTVKRQGLYYIYAQVTFCS.......NREASSQAPFIASLCLKSPG
FasL   KG.GLVINETGLYFVYSKVYFRGQ......SCNNLPLSHKVYMRNSKYPQ
RANKL  NG.KLIVNQDGFYYLYANICFRHHETSGDLATEYLQLMVYVTKTSIKIPS

          E                      F           G
  220        230        240        250        260
Apo2L  PILLMKSARNSCWSKDAE.....YGLYSIYQGGIFELKENDRIFVSVTNE
TNF-β  HVPLLSSQKMVYPGLQE......PWLHSMYHGAAFQLTQGDQLSTHTDGI
TNF-α  KVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRP
CD40L  RFERILLRAANTHSSAKP.....CGQQSIHLGGVFELQPGASVFVNVTDP
FasL   DLVMMEGKMMSYCTTGQ......MWARSSYLGAVFNLTSADHLYVNVSEL
RANKL  SHTLMKGGSTKYWSGNSE.....FHFYSINVGGFFKLRSGEEISIEVSNP

              H
        270        280
Apo2L  HLIDMDHE.ASFFGAFLVG
TNF-β  PHLVLSPS.TVFFGAFAL.
TNF-α  DYLLFAESGQVYFGIIAL.
CD40L  SQVSHGTG.FTSFGLLKL.
FasL   SLVNFEES.QTFFGLYK..
RANKL  SLLDPDQD.ATYFGAFKVR
```

FIG._5

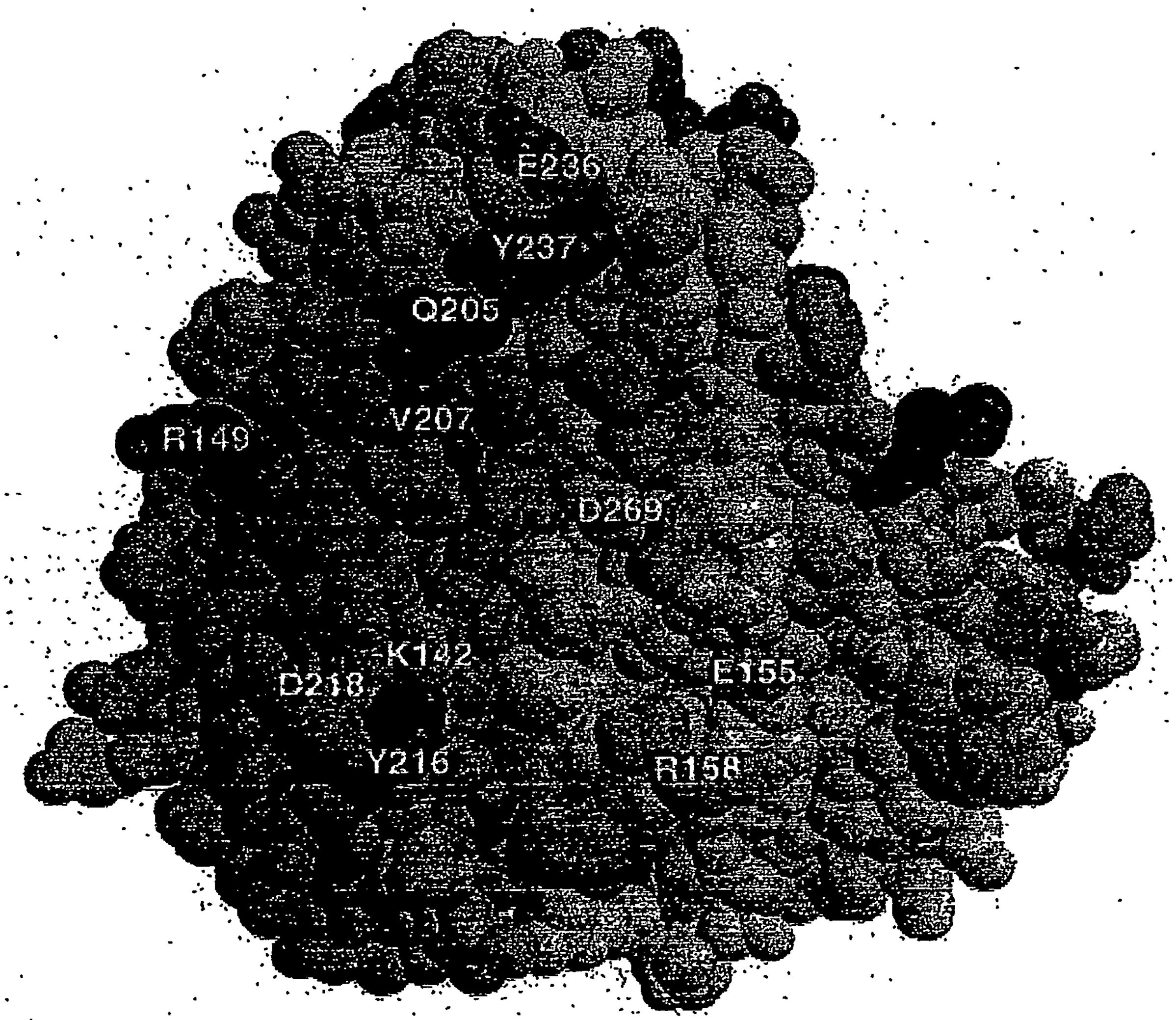
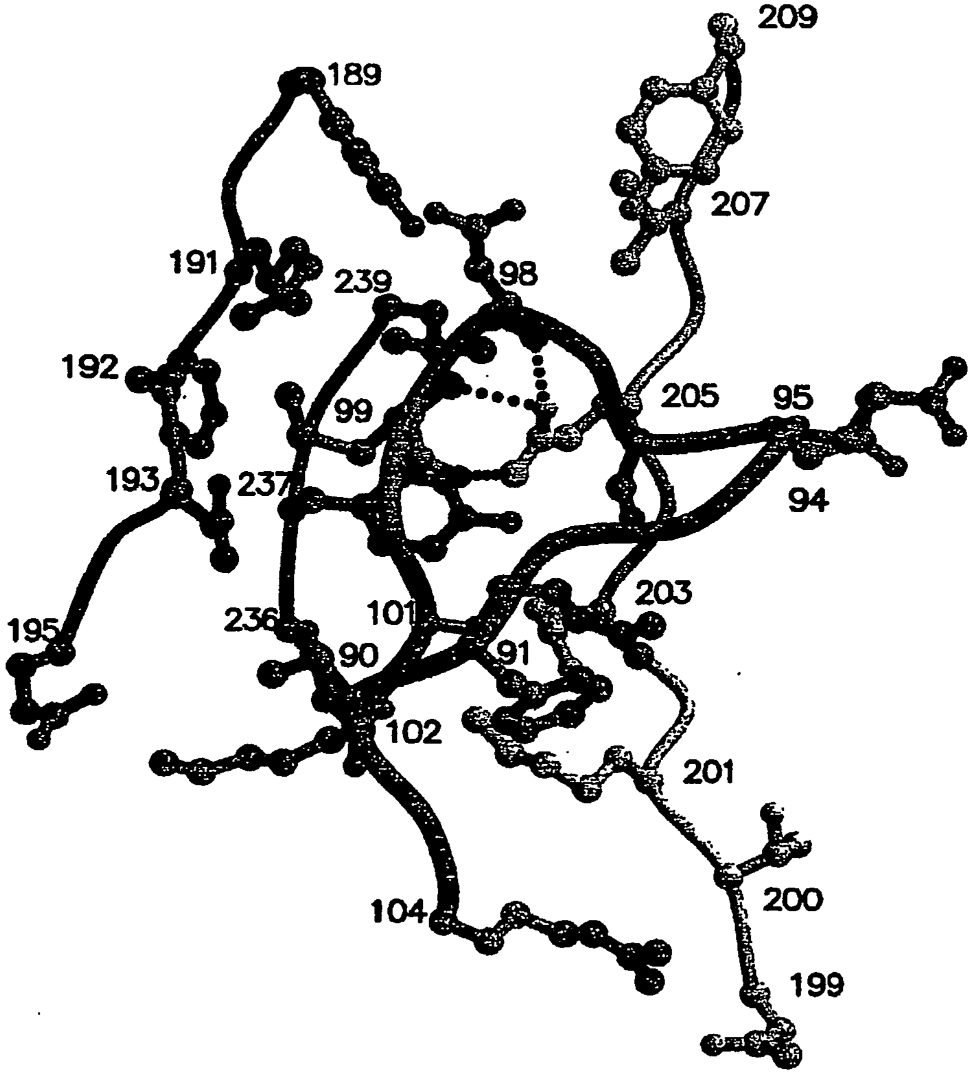


FIG._6

Apo2L • DR5 Patch A



Receptor Sequences:

DR5 ^{90}TFREEDSPEMCRKCR^{104}
DR4 TFRNDNSAEMCRKCS

Apo2L = Dark Shading
DR5 = Light Shading

FIG._7A

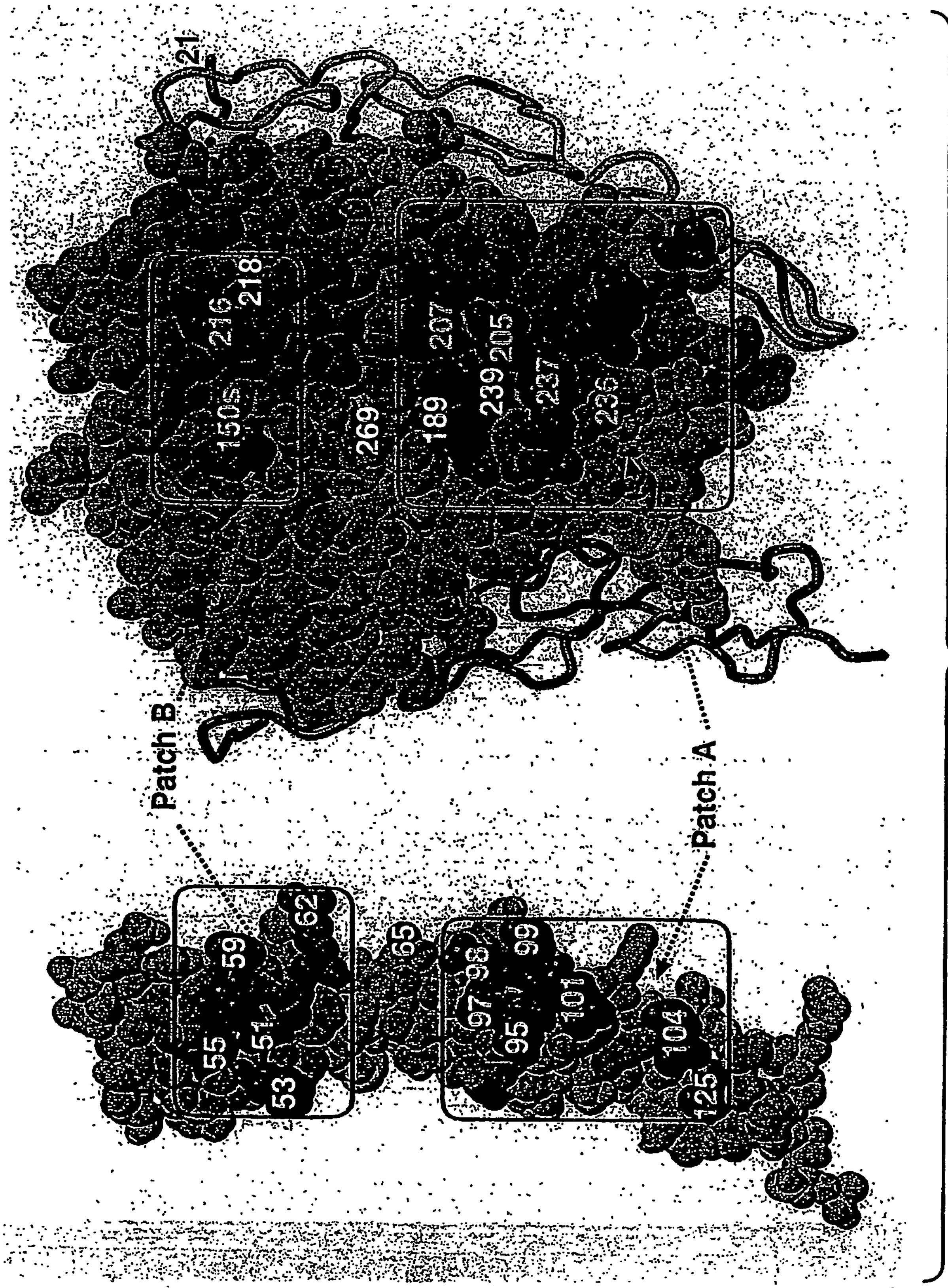

Patch B
Patch A
21
216
218
207
205
150s
269
189
239
237
236
55
51
59
62
53
65
97
98
99
95
101
104
125

FIG._7B

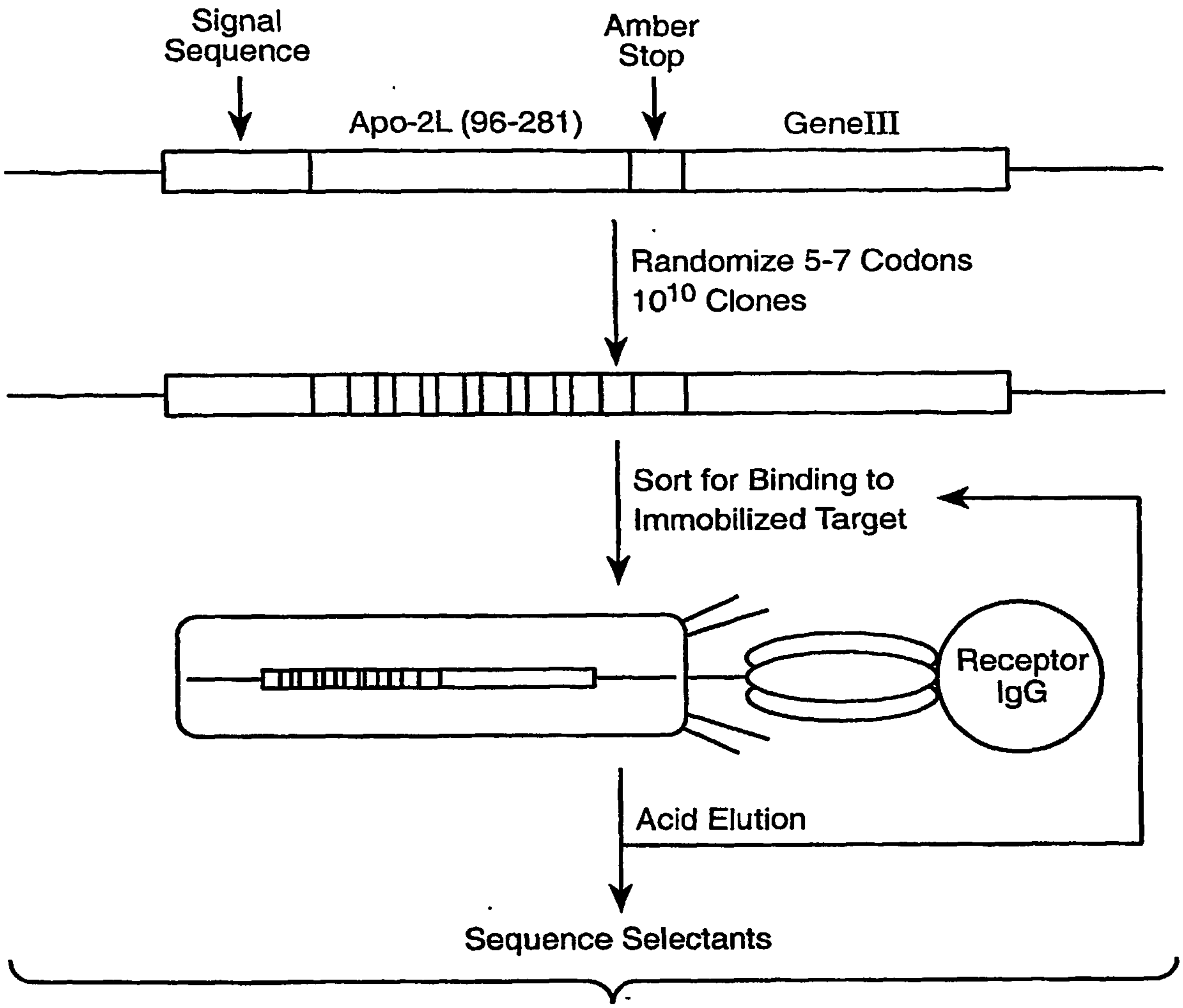


FIG._8A

Apo-2L Phage Display Libraries

	DR5	DR4
199LB		
Y189	E98	E98
R191	M99	M99
Q193	T90	T90
N199	R104	S104
K201	R101	R101
Y209	E98	E98

• Hard Randomize Libraries: Sort Against DR4 or DR5 +/- Competitor.

FIG._8B

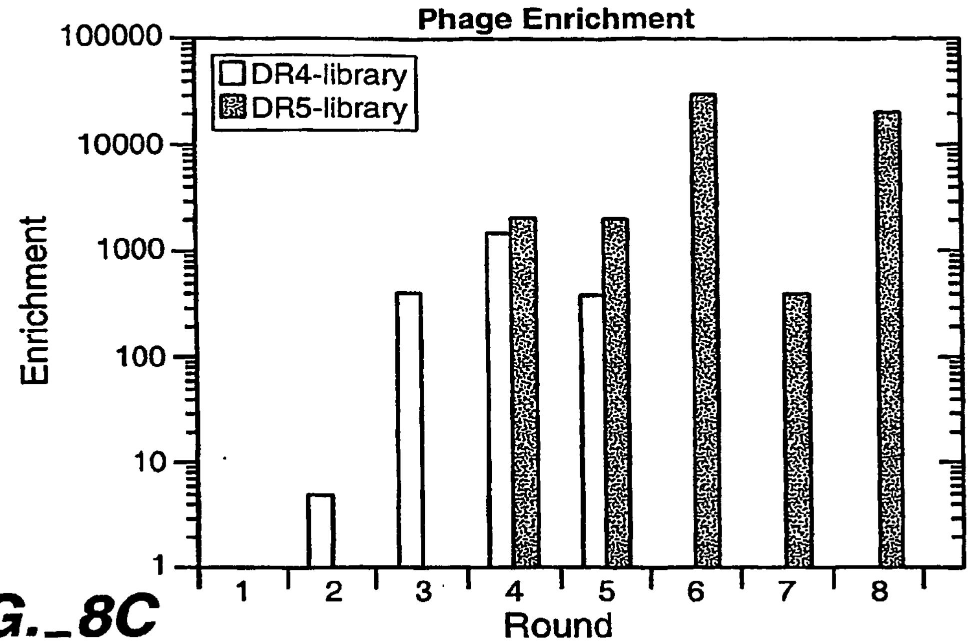

Phage Enrichment
DR4-library
DR5-library
Enrichment
100000
10000
1000
100
10
1
1
2
3
4
5
6
7
8
Round

FIG._8C

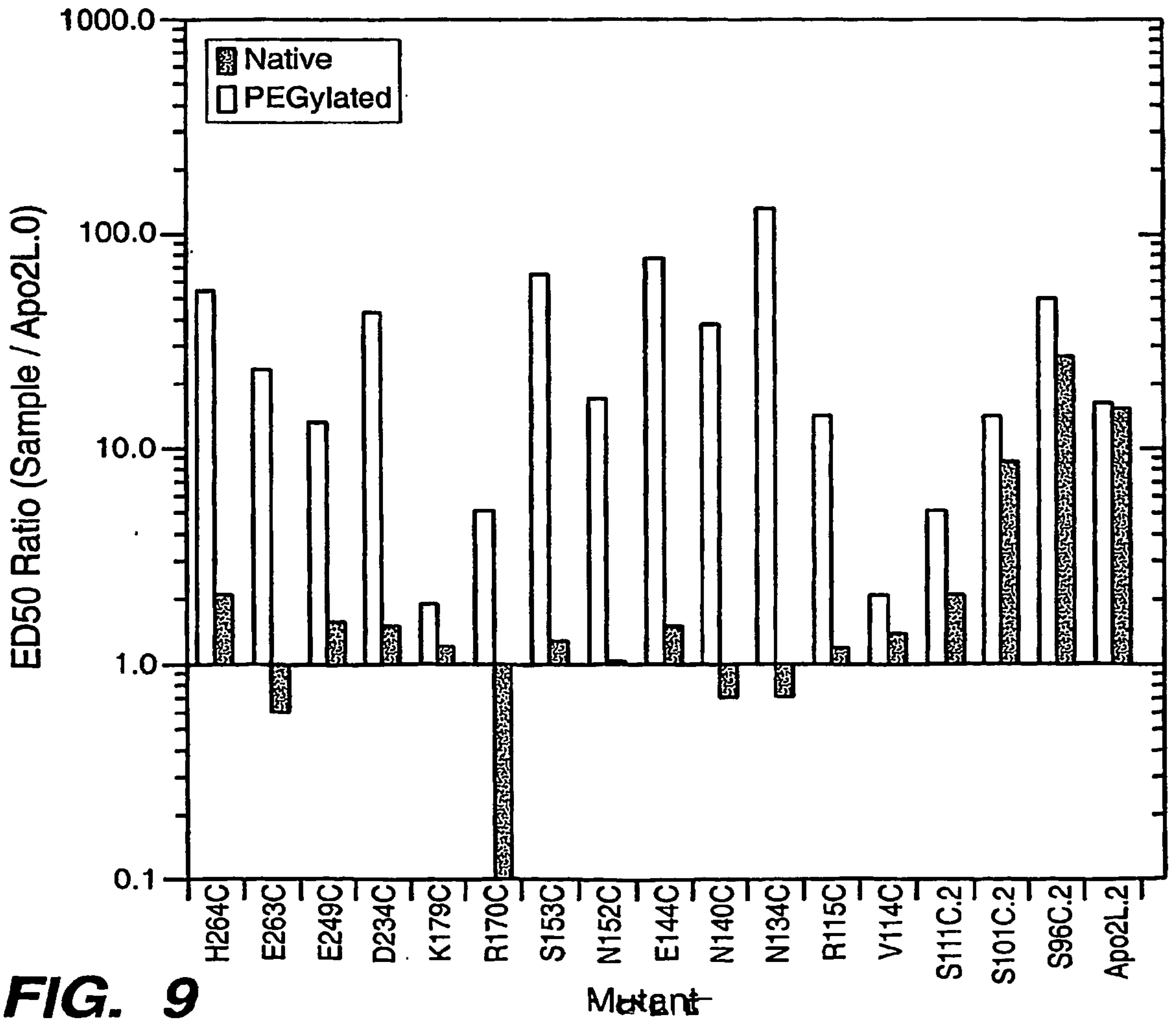

Native
PEGylated
ED50 Ratio (Sample / Apo2L.0)
1000.0
100.0
10.0
1.0
0.1
H264C
E263C
E249C
D234C
K179C
R170C
S153C
N152C
E144C
N140C
N134C
R115C
V114C
S111C.2
S101C.2
S96C.2
Apo2L.2
Mutant

FIG. 9

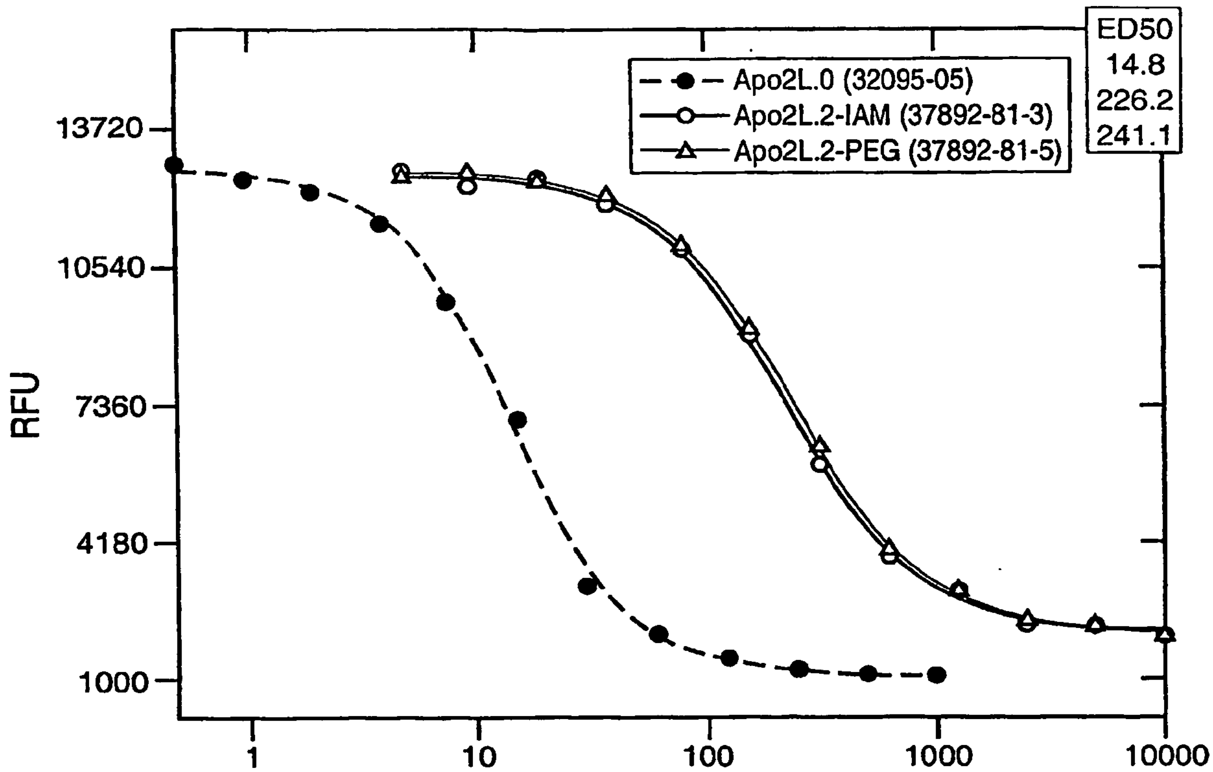


FIG._10A

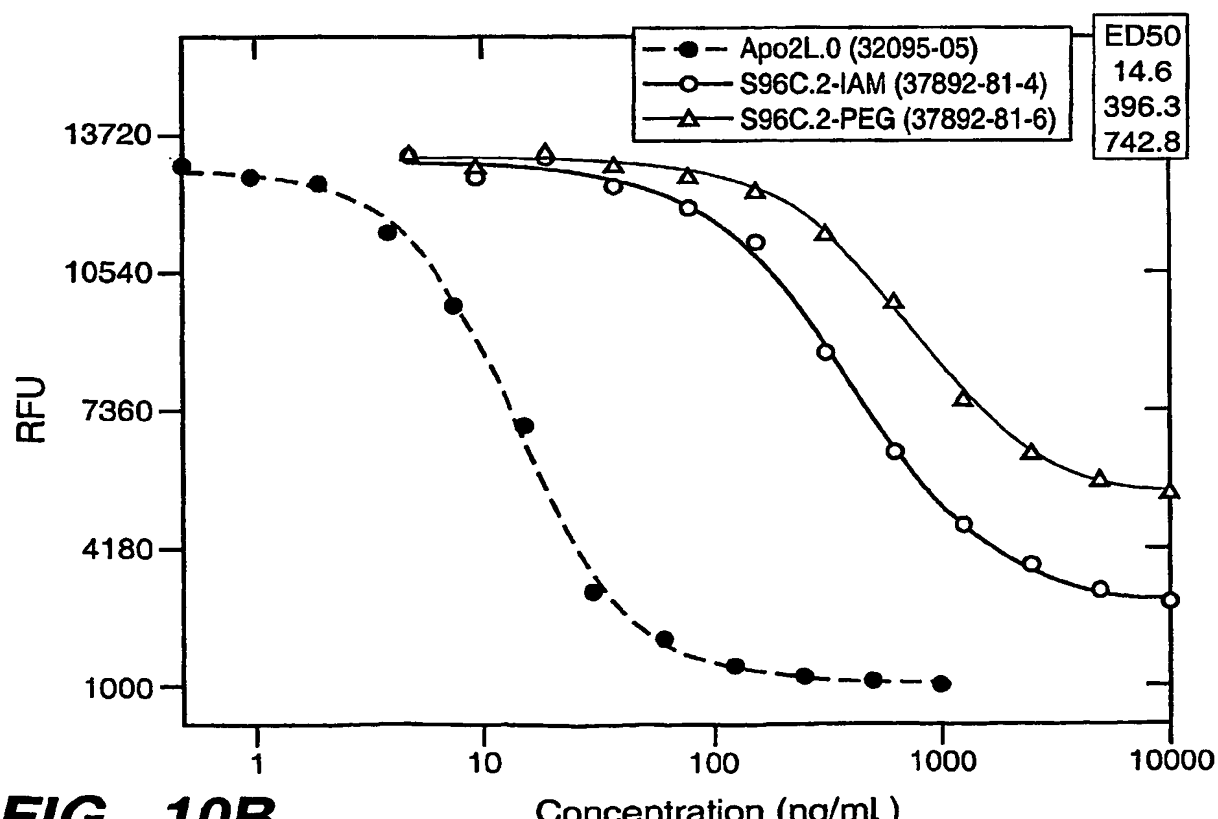


FIG._10B

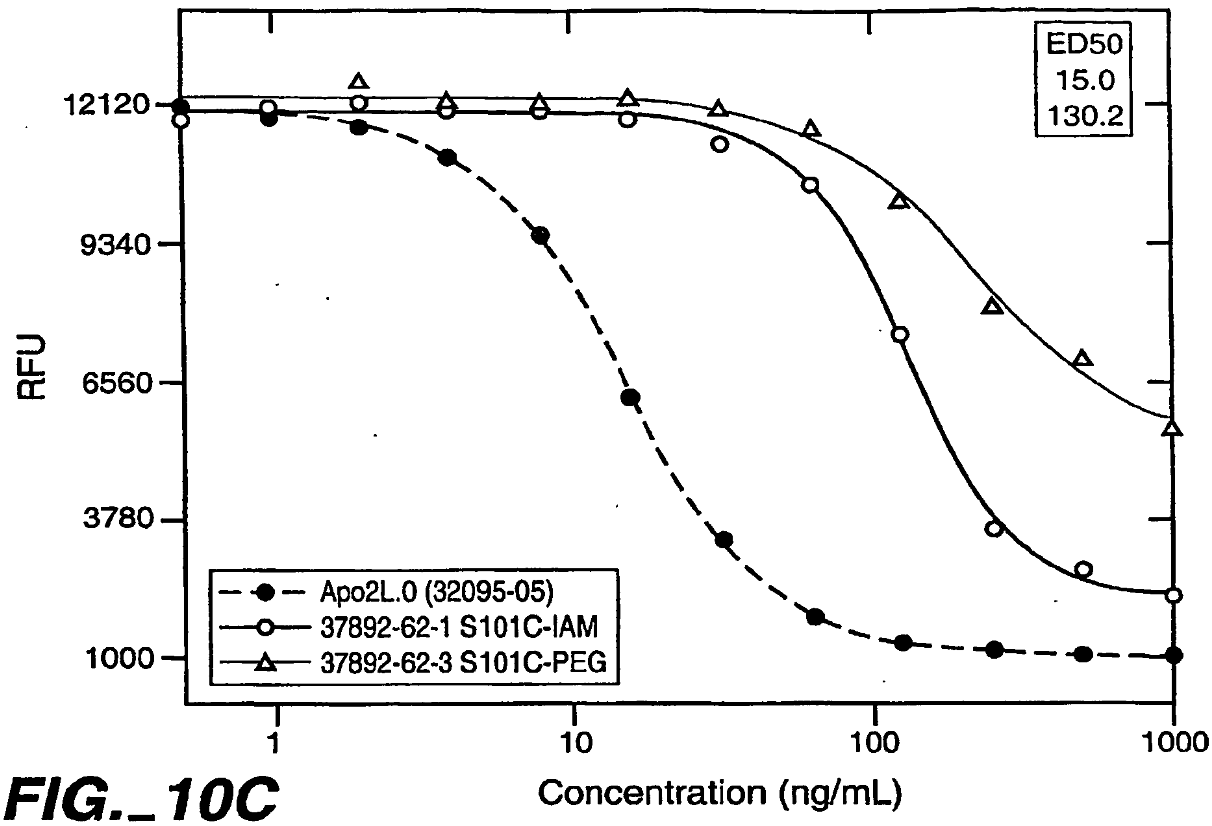


FIG._10C

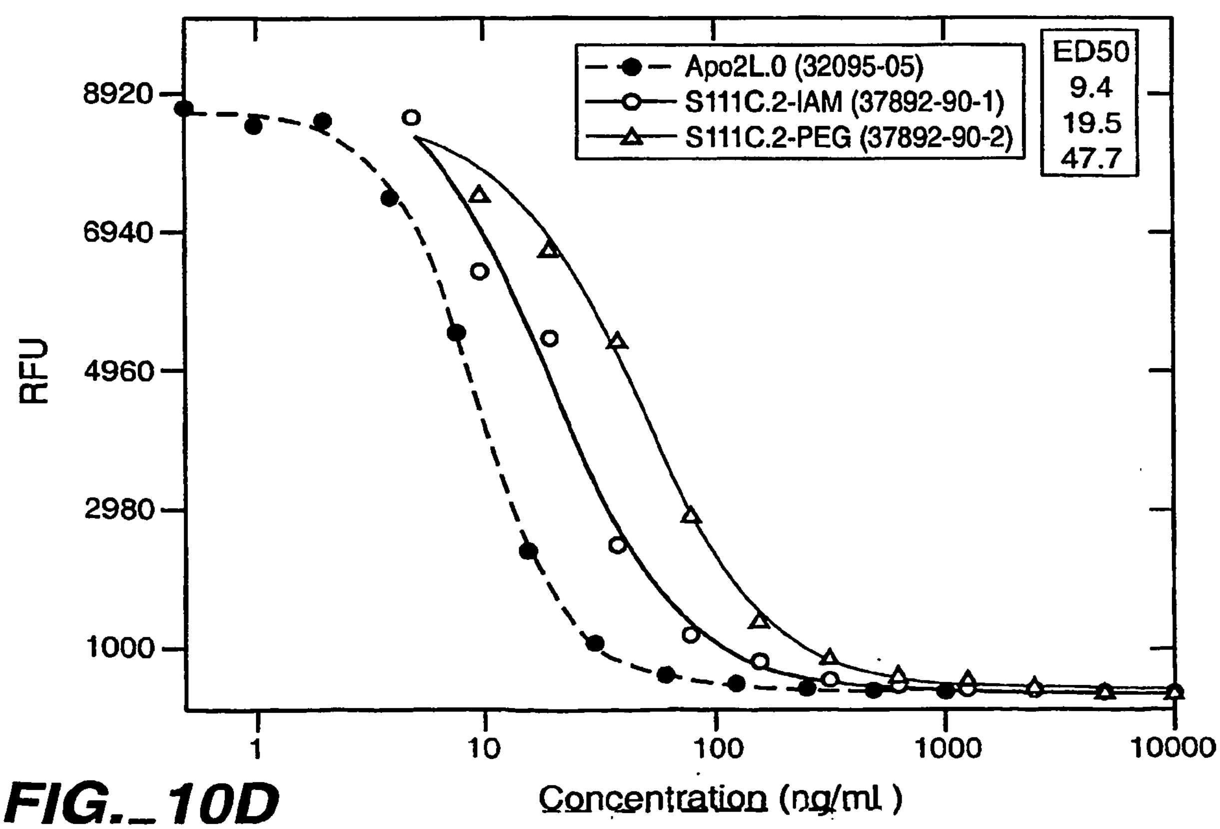


FIG._10D

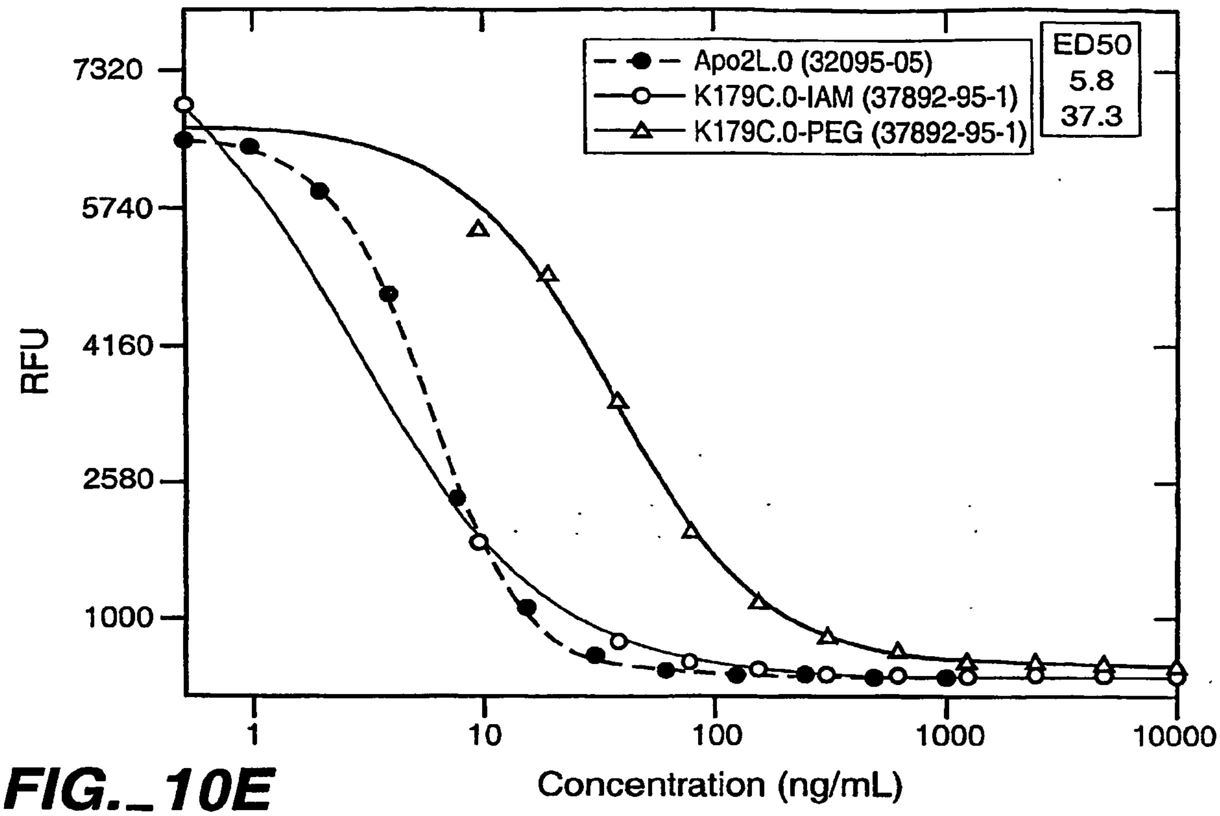


FIG._10E

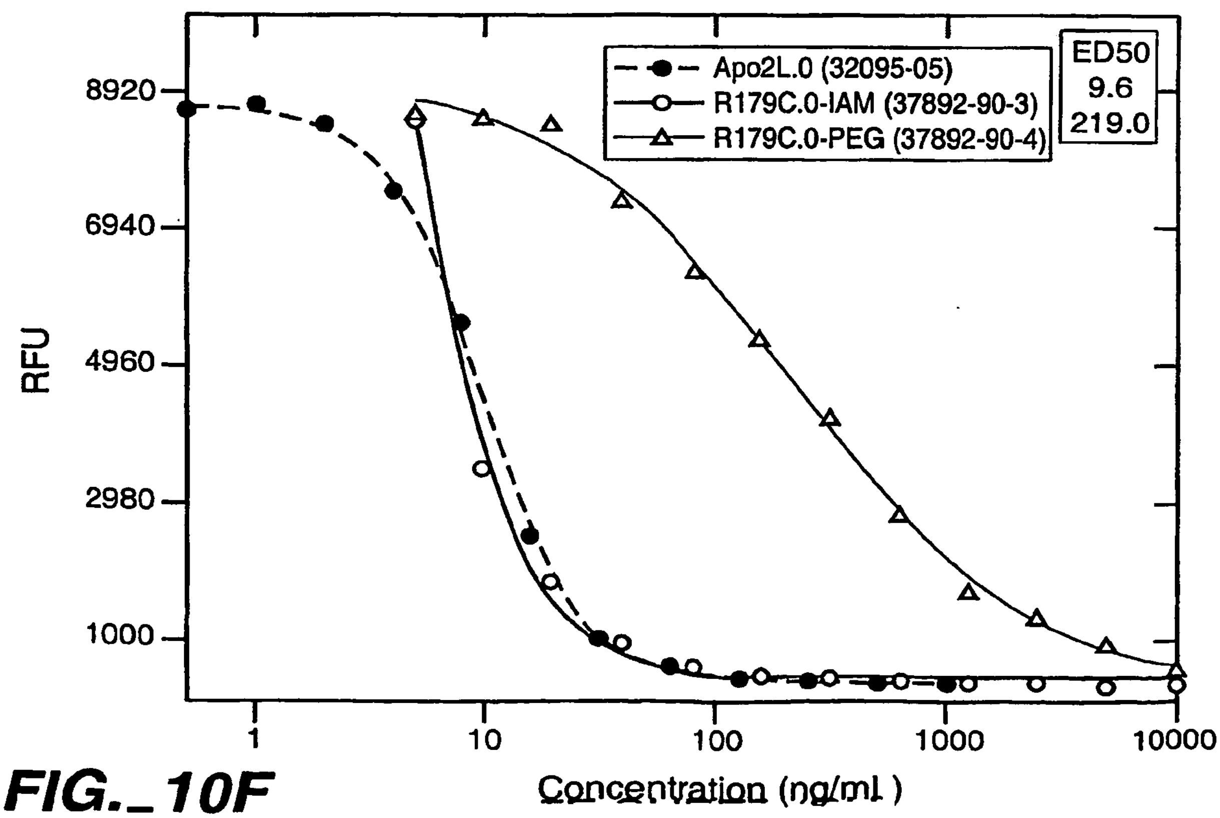


FIG._10F

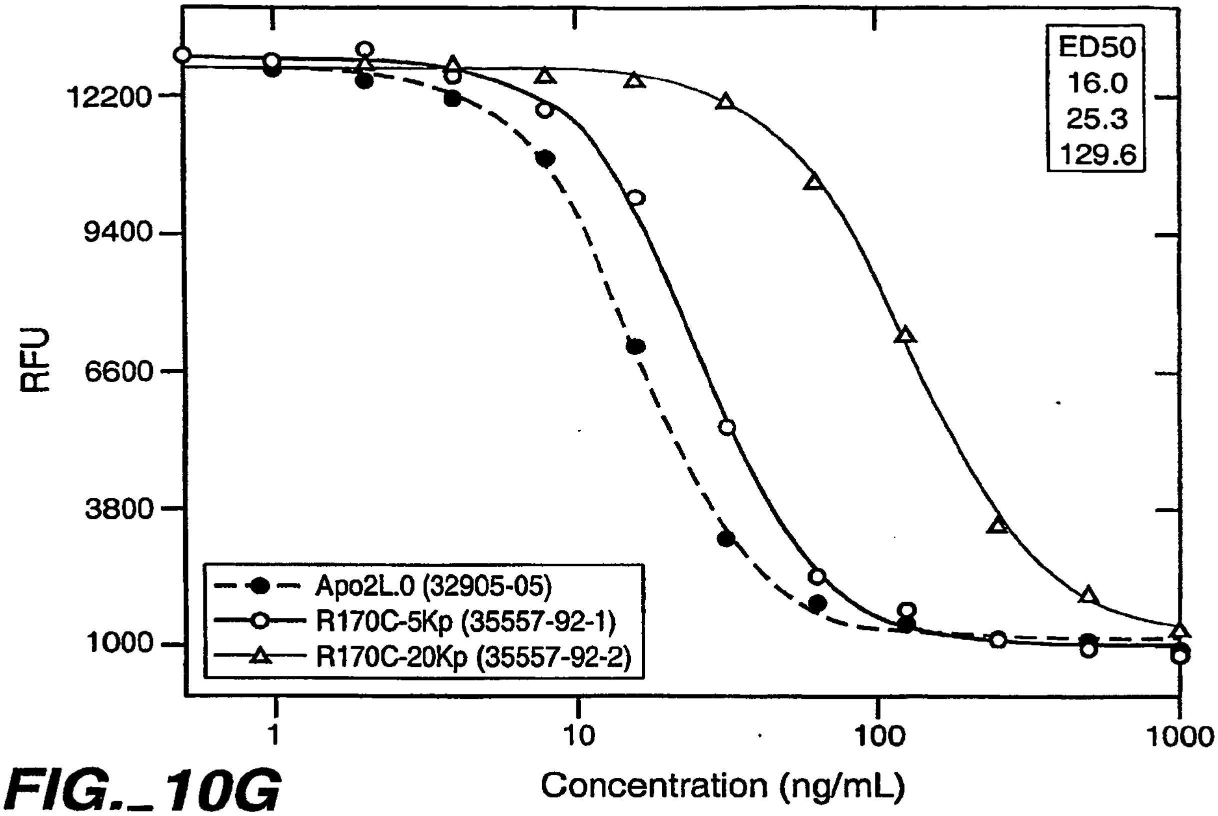


FIG._10G

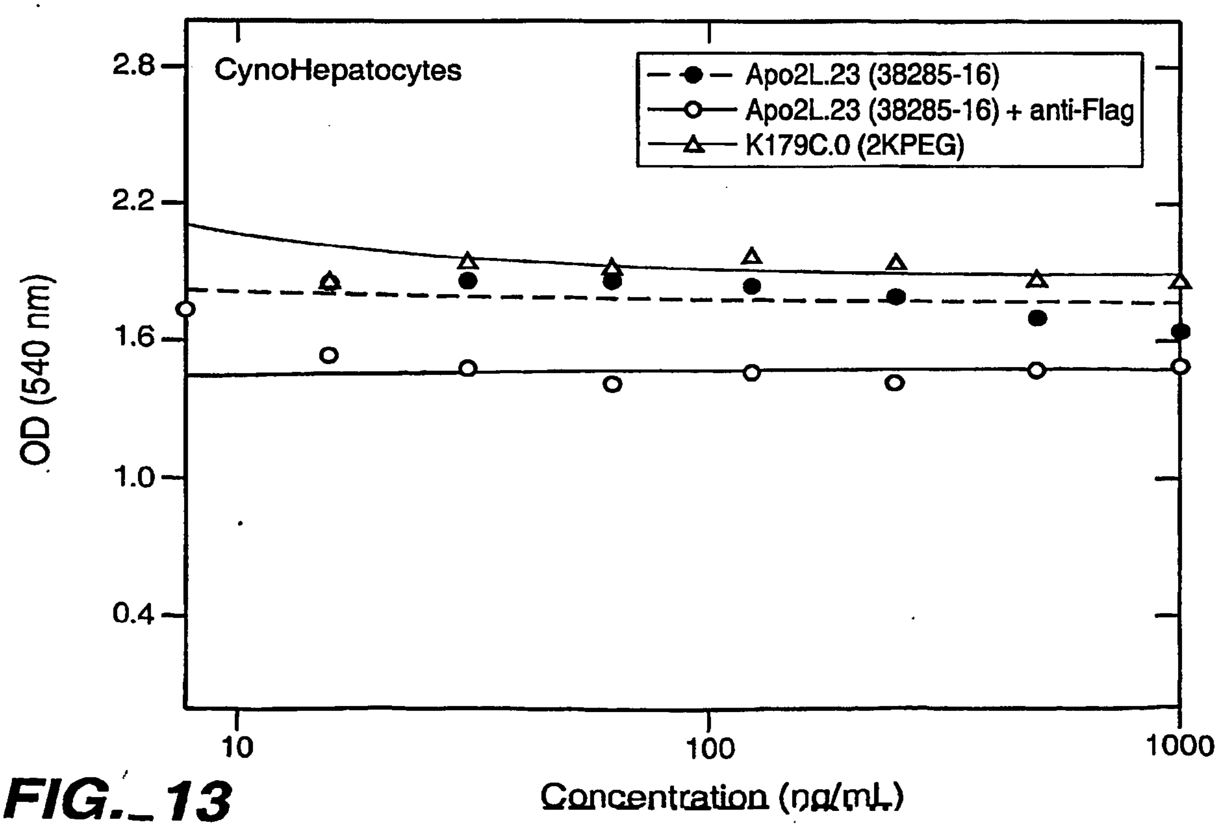


FIG._13

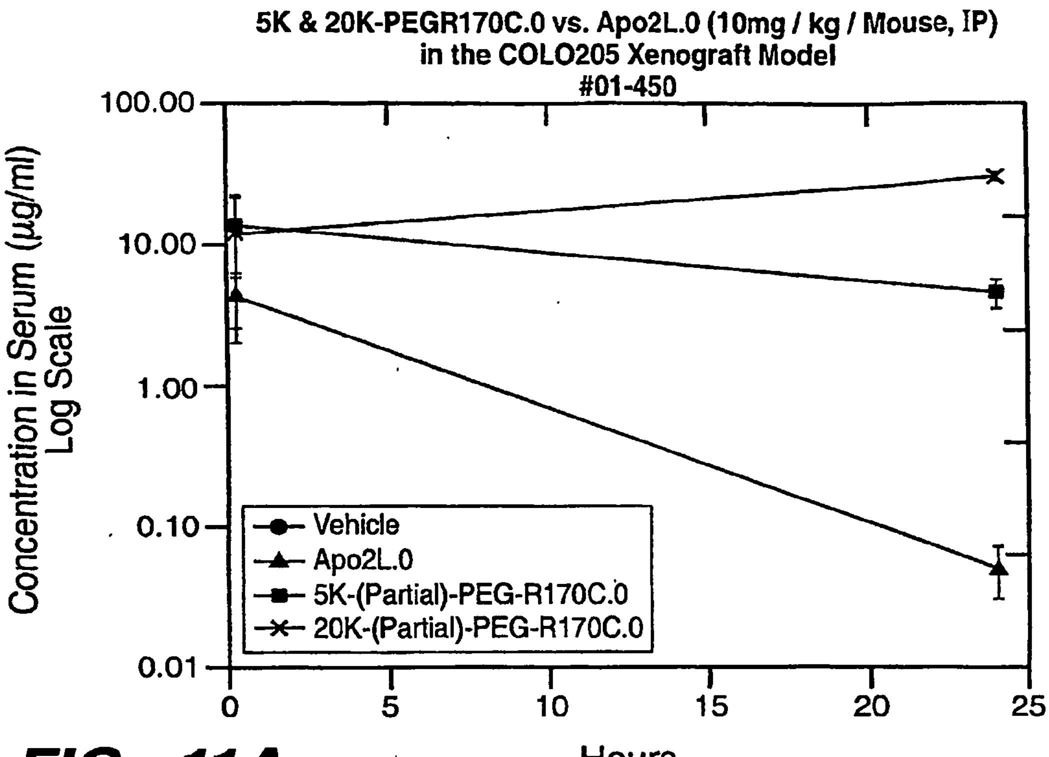


FIG._11A

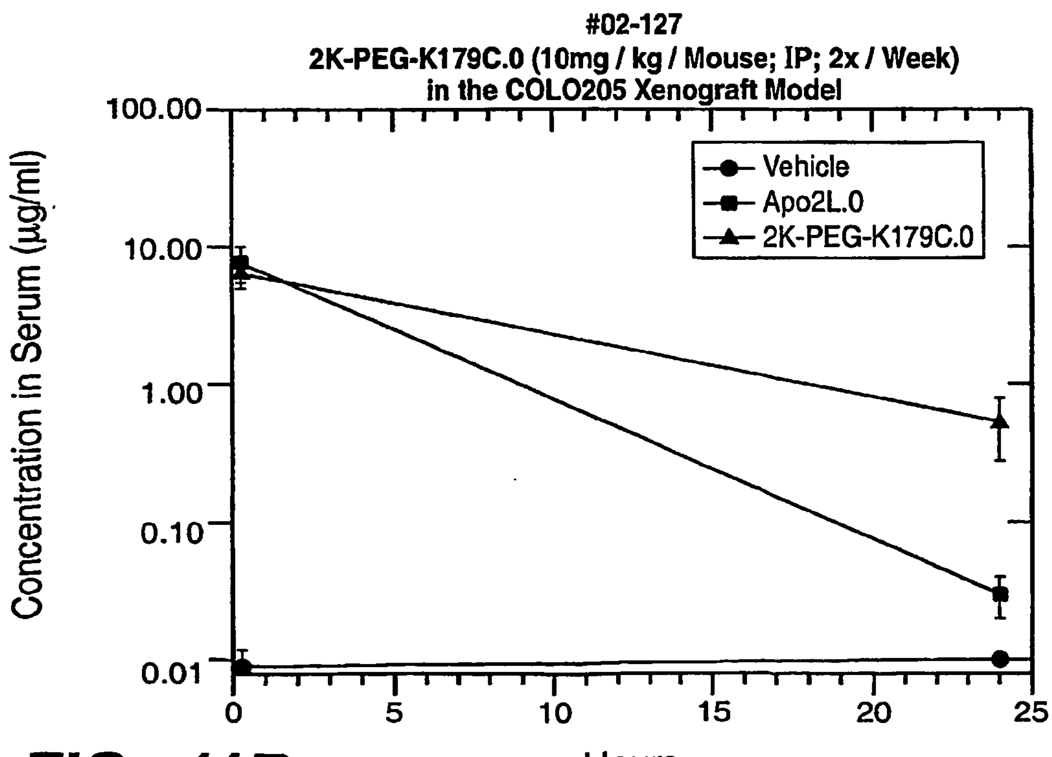


FIG._11B

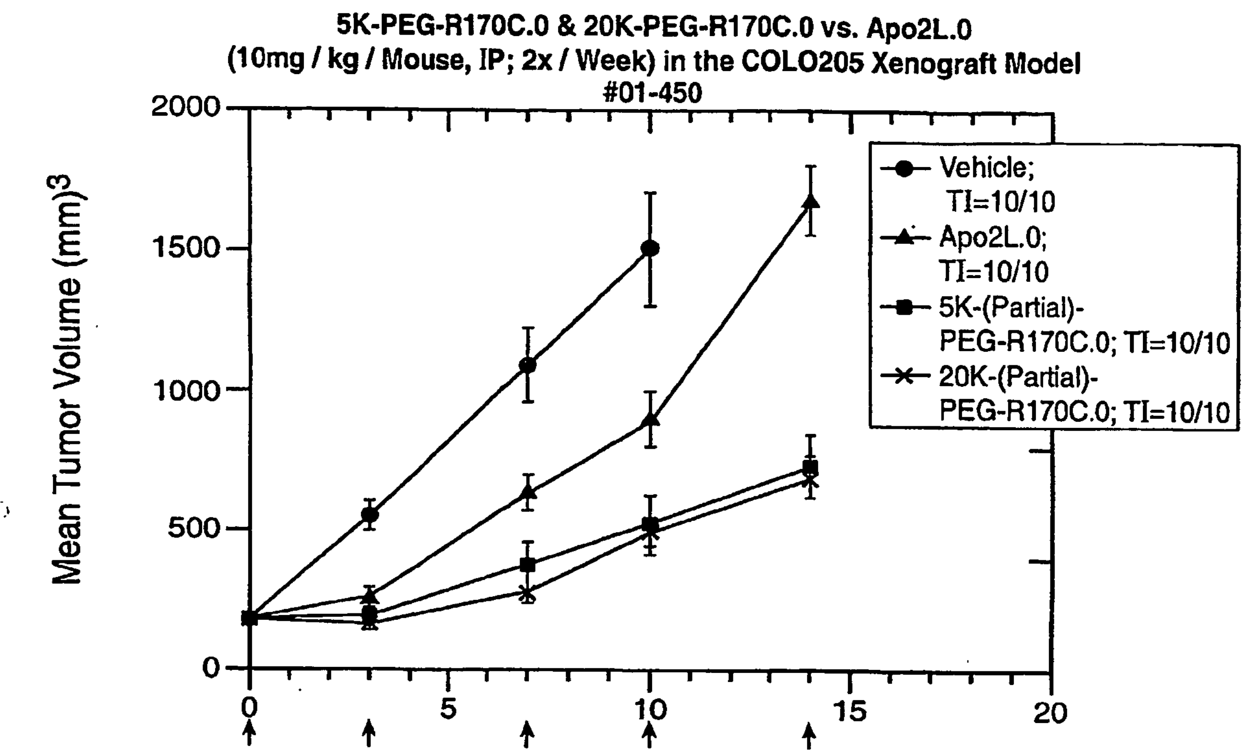


FIG._12A

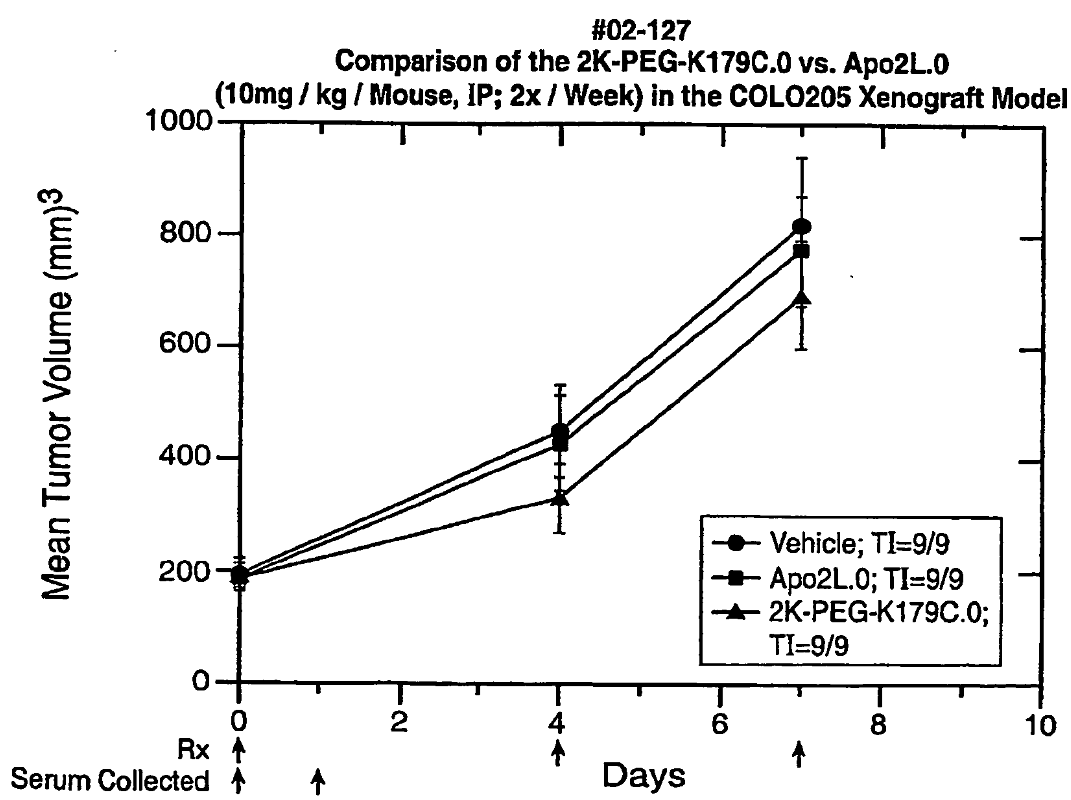


FIG._12B

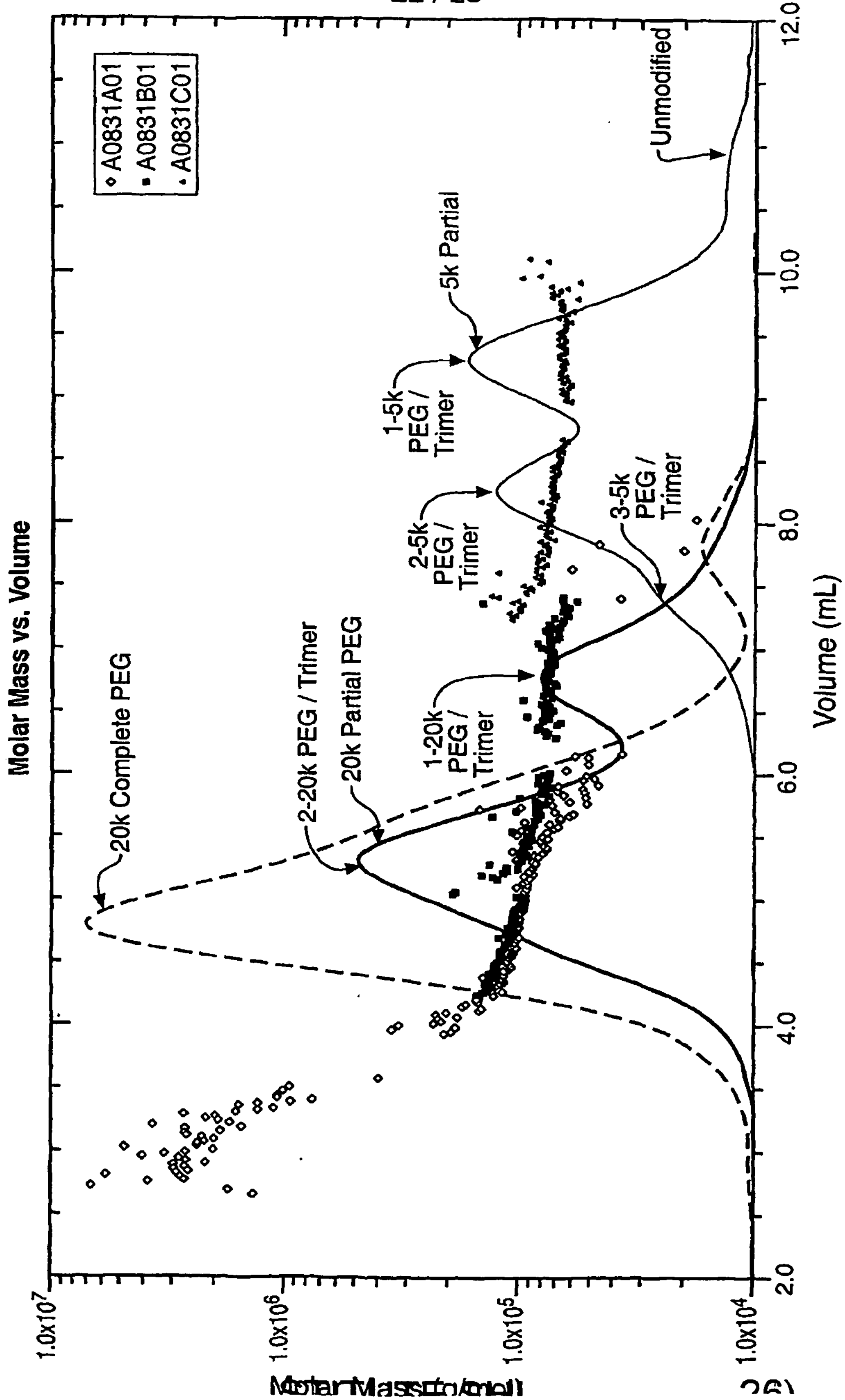

Molar Mass vs. Volume
A0831A01
A0831B01
A0831C01
20k Complete PEG
2-20k PEG / Trimer
20k Partial PEG
1-20k PEG / Trimer
2-5k PEG / Trimer
3-5k PEG / Trimer
1-5k PEG / Trimer
5k Partial
Unmodified
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
2.0
4.0
6.0
8.0
10.0
12.0
Volume (mL)

FIG._14

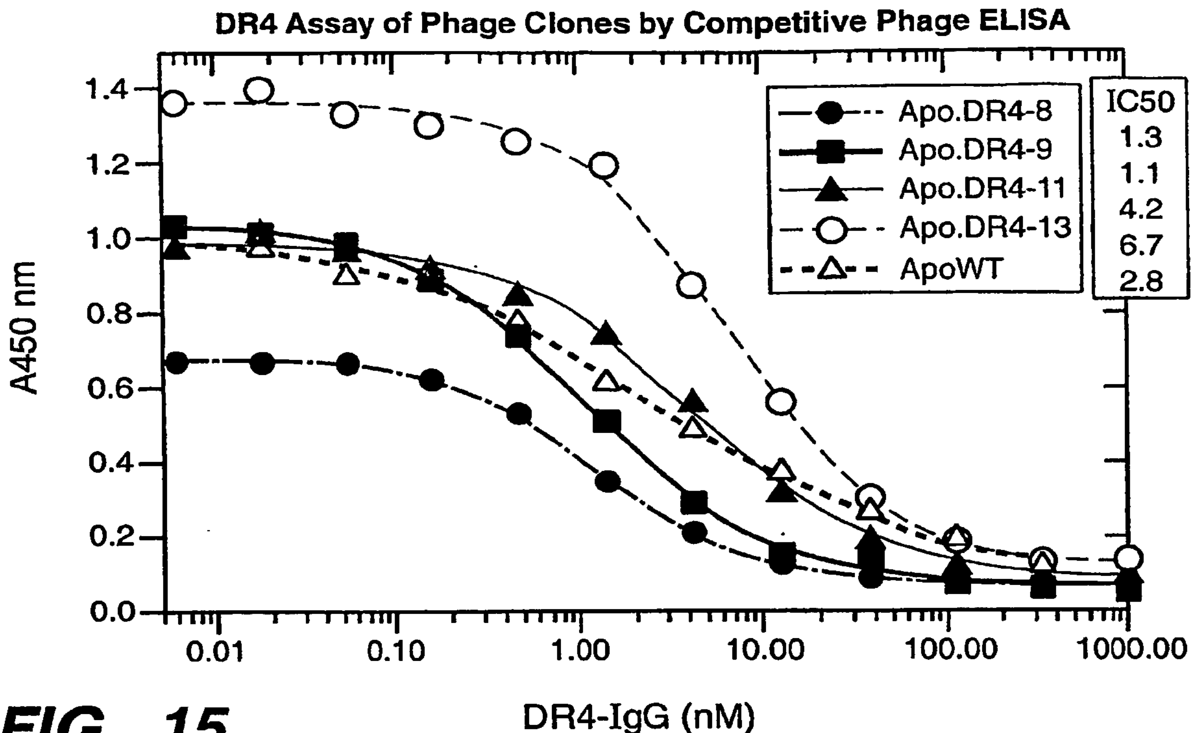

DR4 Assay of Phage Clones by Competitive Phage ELISA
A450 nm
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Apo.DR4-8
Apo.DR4-9
Apo.DR4-11
Apo.DR4-13
ApoWT
IC50
1.3
1.1
4.2
6.7
2.8
0.01
0.10
1.00
10.00
100.00
1000.00
DR4-IgG (nM)

FIG._15

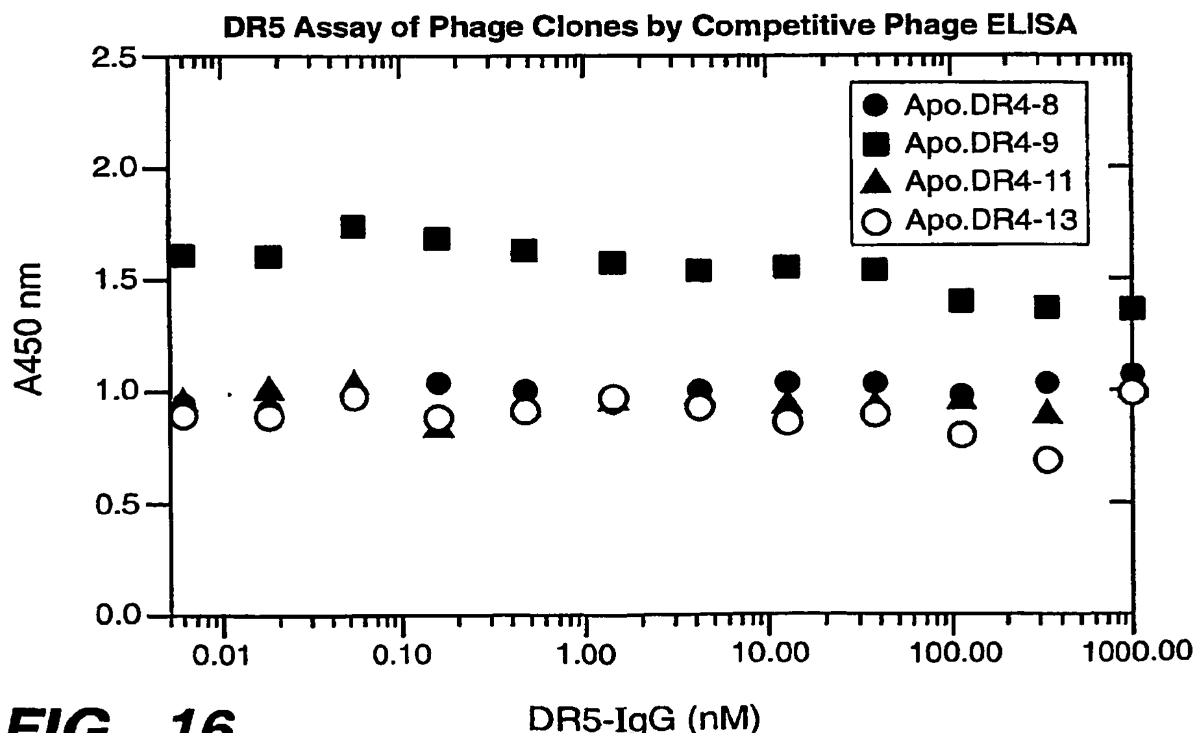

DR5 Assay of Phage Clones by Competitive Phage ELISA
A450 nm
2.5
2.0
1.5
1.0
0.5
0.0
Apo.DR4-8
Apo.DR4-9
Apo.DR4-11
Apo.DR4-13
0.01
0.10
1.00
10.00
100.00
1000.00
DR5-IgG (nM)

FIG._16

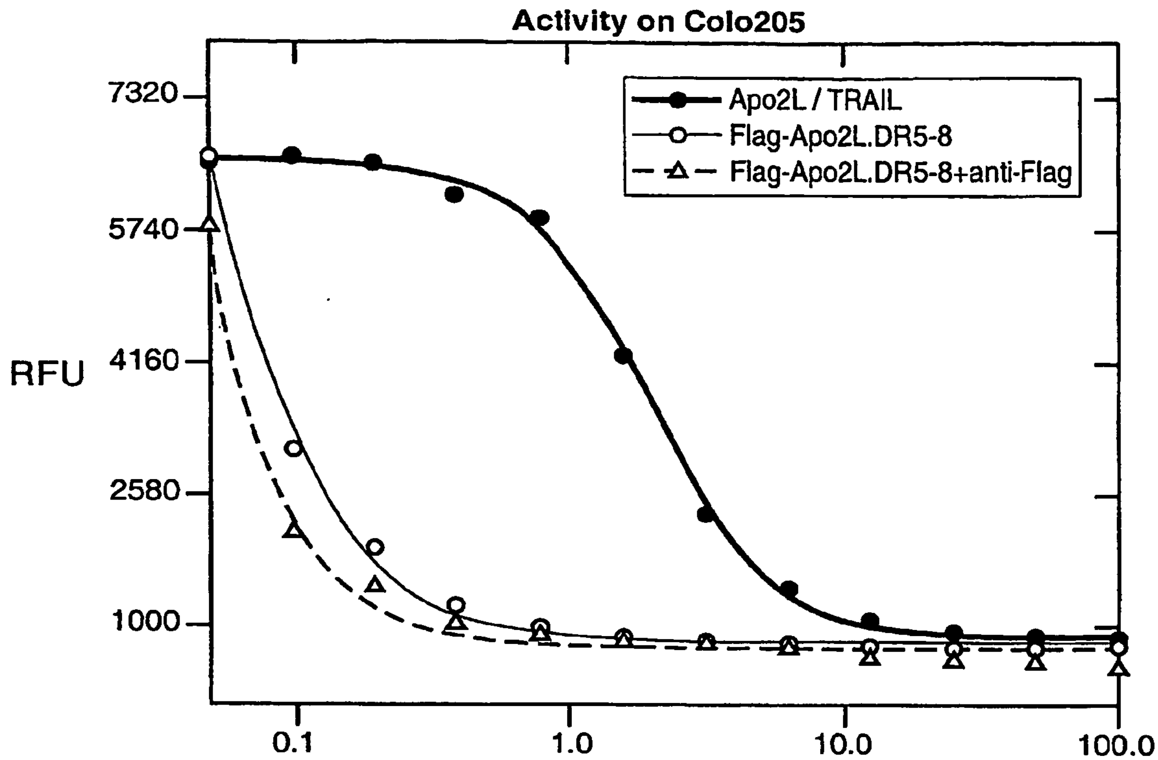


FIG._17A

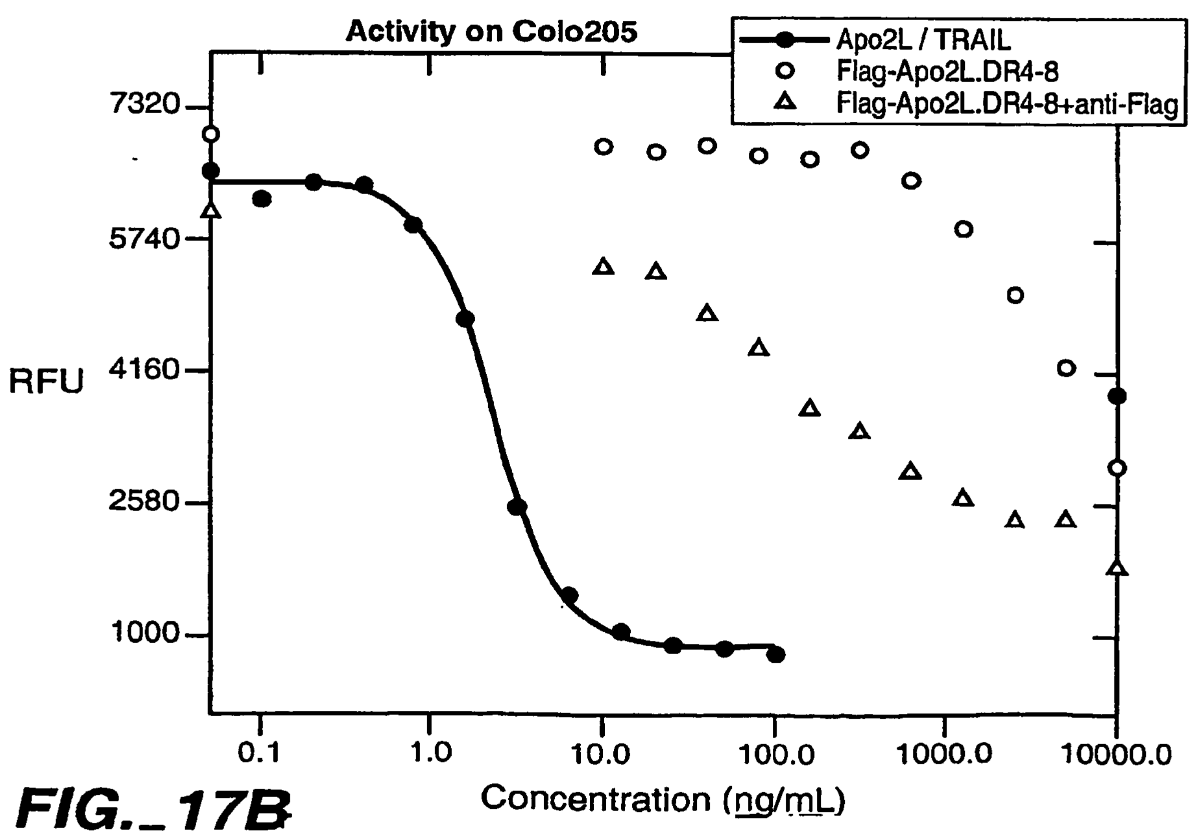


FIG._17B

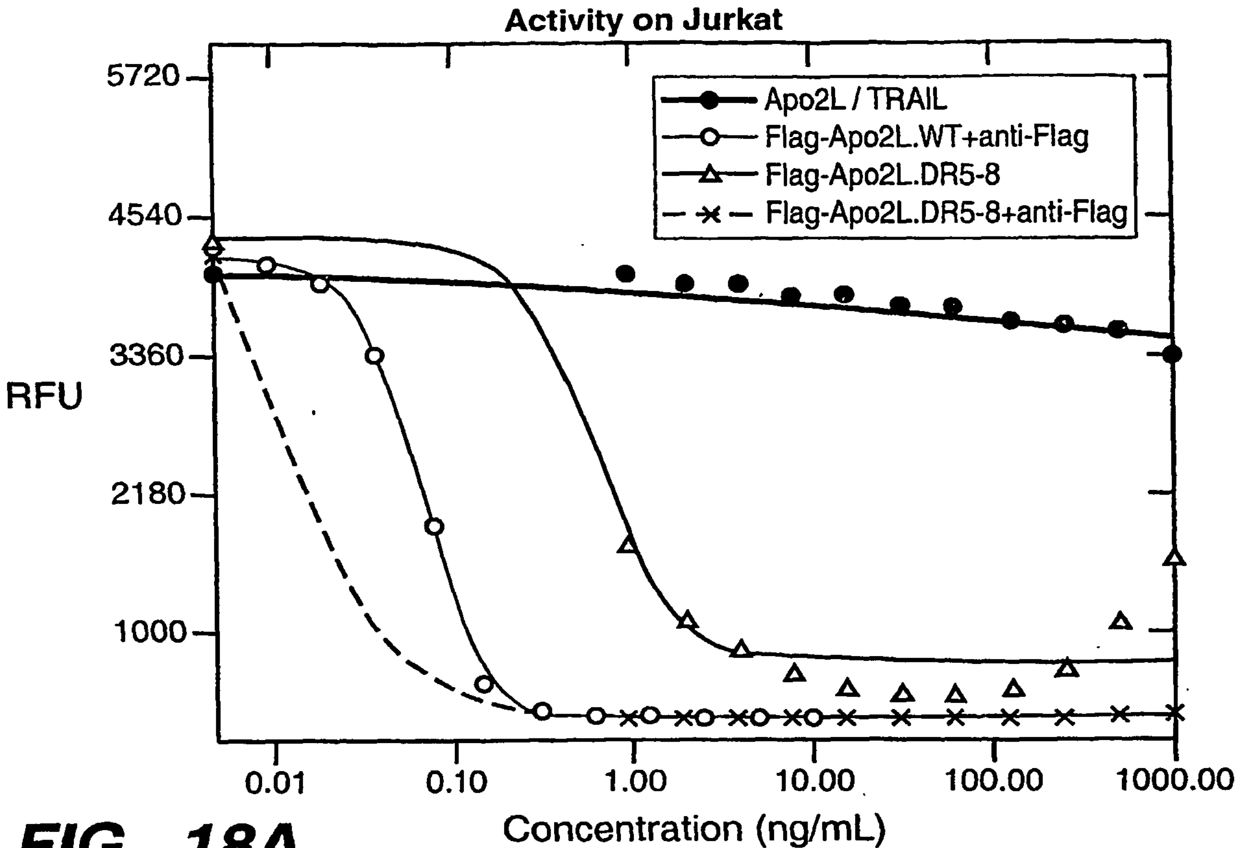


FIG._18A

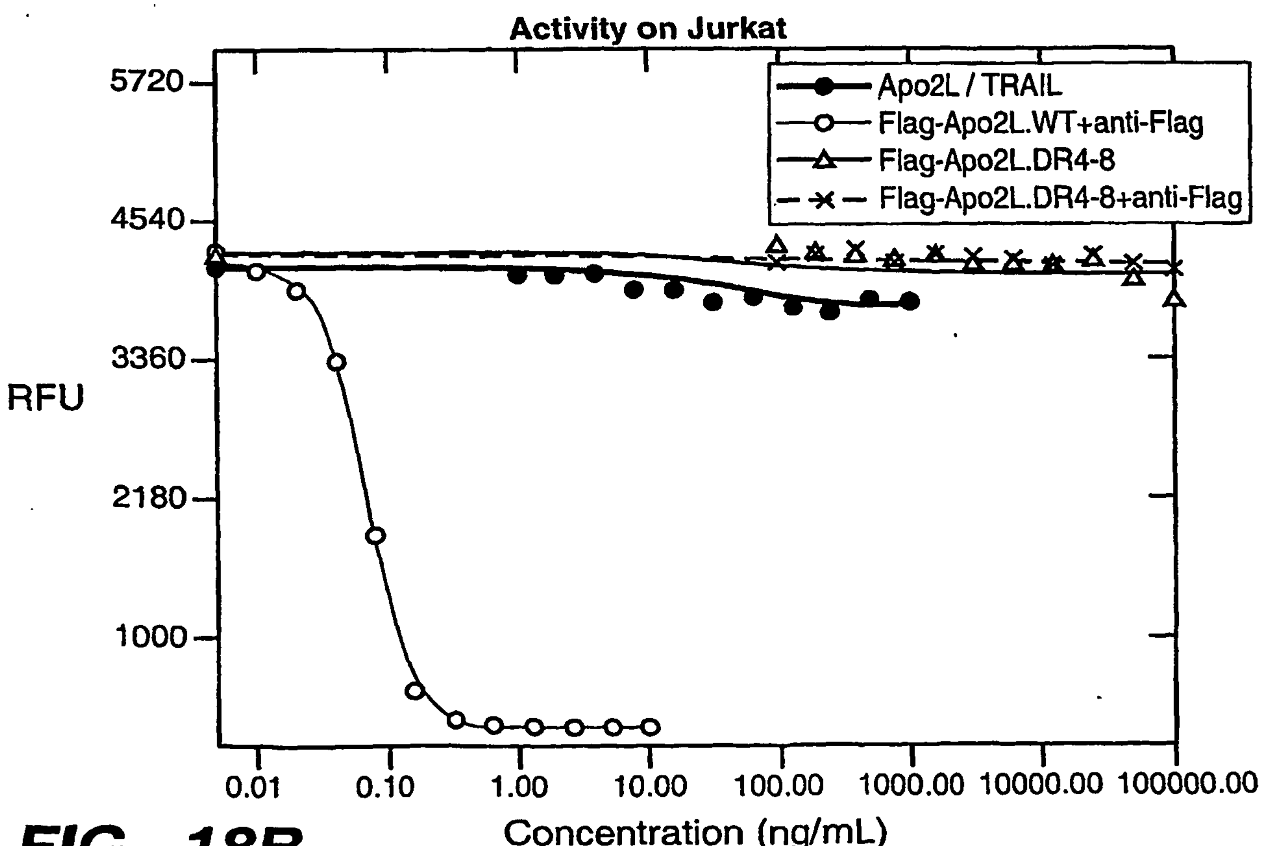


FIG._18B

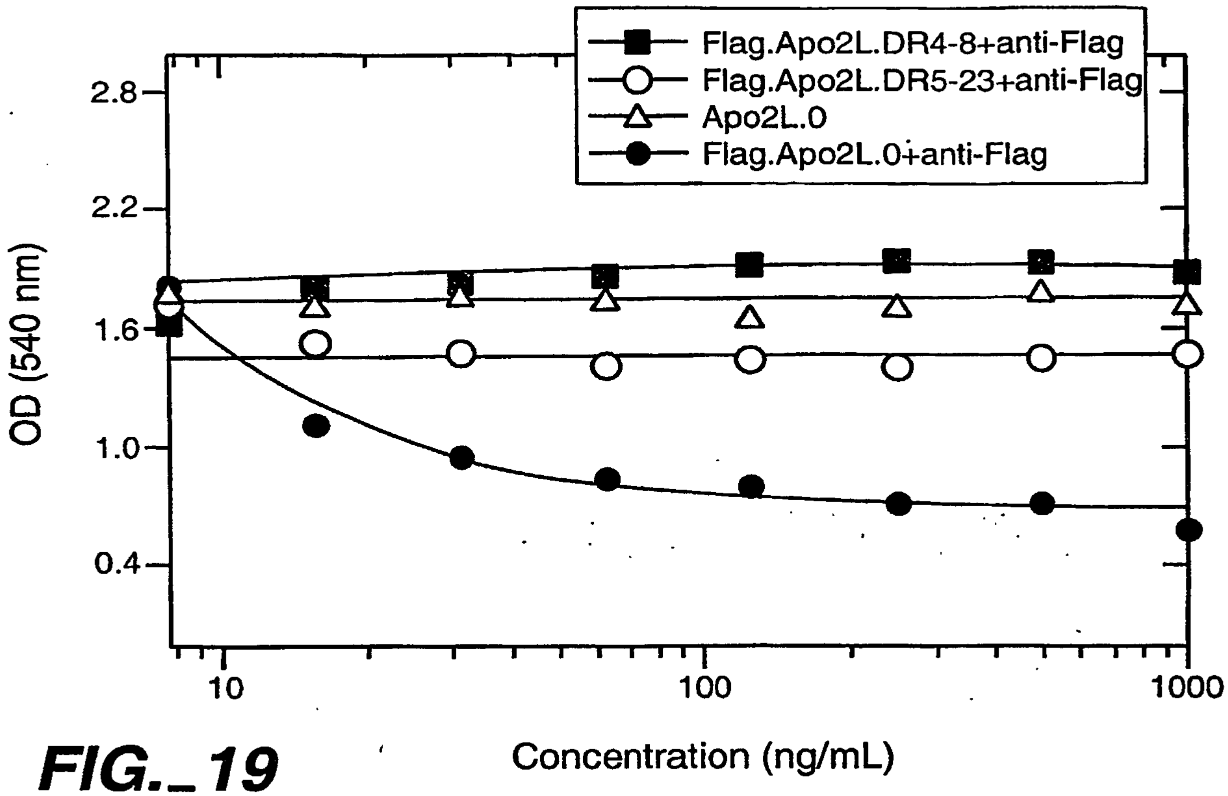
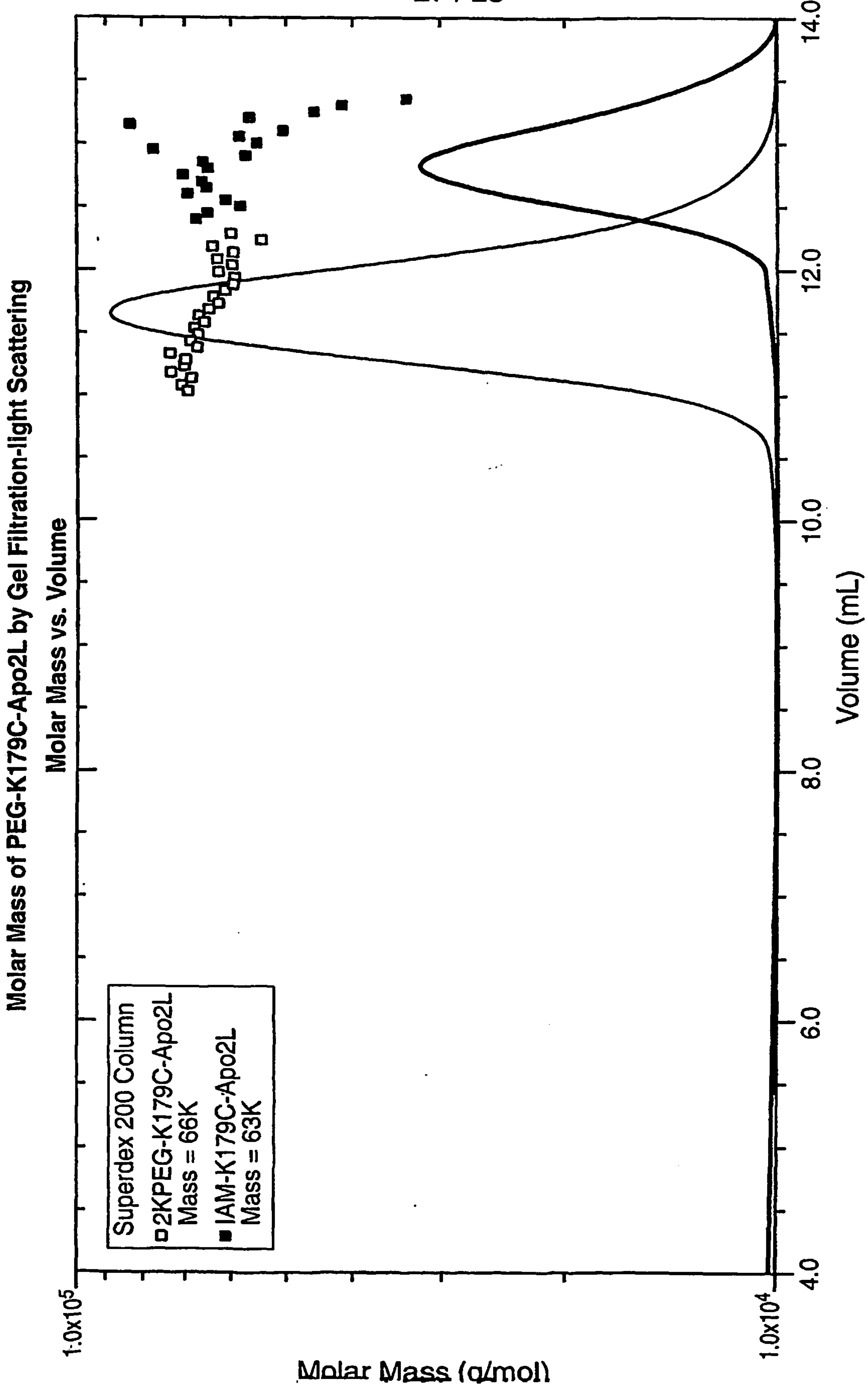

Flag.Apo2L.DR4-8+anti-Flag
Flag.Apo2L.DR5-23+anti-Flag
Apo2L.0
Flag.Apo2L.0+anti-Flag
2.8
2.2
1.6
1.0
0.4
OD (540 nm)
10
100
1000
Concentration (ng/mL)

FIG._19



Molar Mass of PEG-K179C-Apo2L by Gel Filtration-light Scattering
Molar Mass vs. Volume
Superdex 200 Column
2KPEG-K179C-Apo2L
Mass = 66K
IAM-K179C-Apo2L
Mass = 63K
1.0x10^5
1.0x10^4
Molar Mass (g/mol)
4.0
6.0
8.0
10.0
12.0
14.0
Volume (mL)

FIG._20

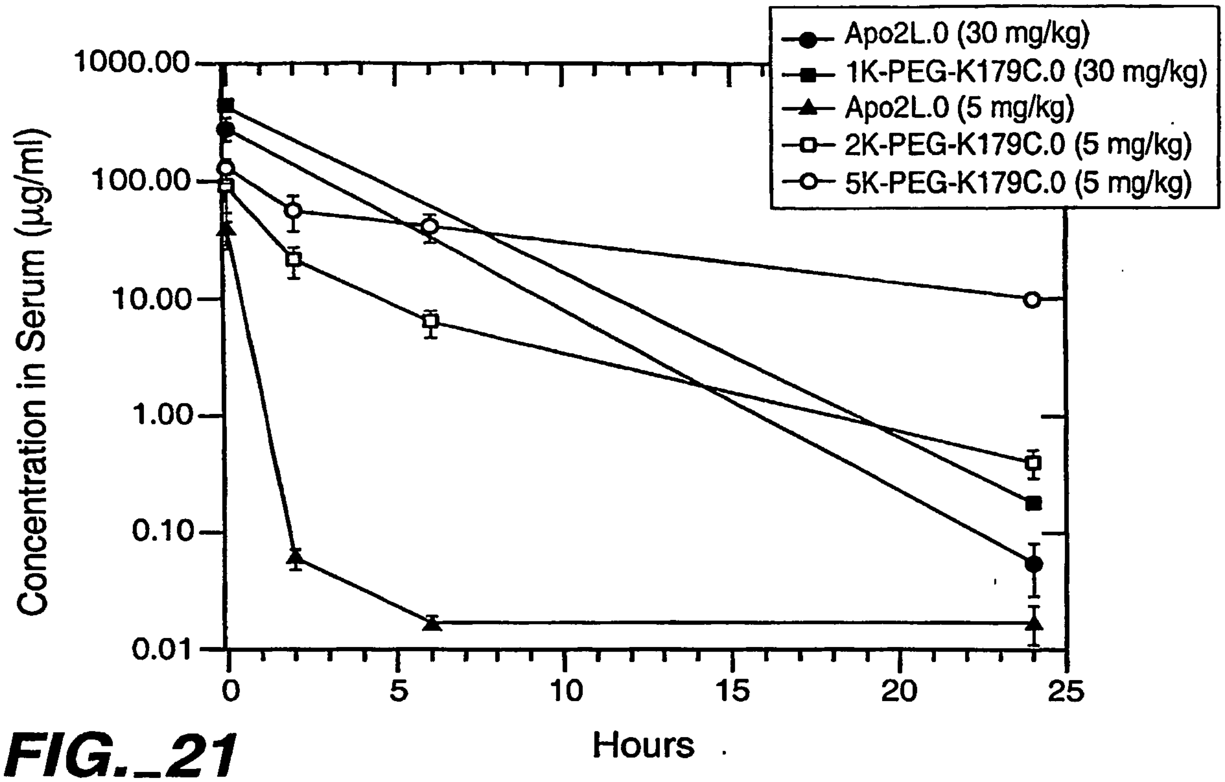

Apo2L.0 (30 mg/kg)
1K-PEG-K179C.0 (30 mg/kg)
Apo2L.0 (5 mg/kg)
2K-PEG-K179C.0 (5 mg/kg)
5K-PEG-K179C.0 (5 mg/kg)
Concentration in Serum (µg/ml)
1000.00
100.00
10.00
1.00
0.10
0.01
0
5
10
15
20
25
Hours

FIG._21

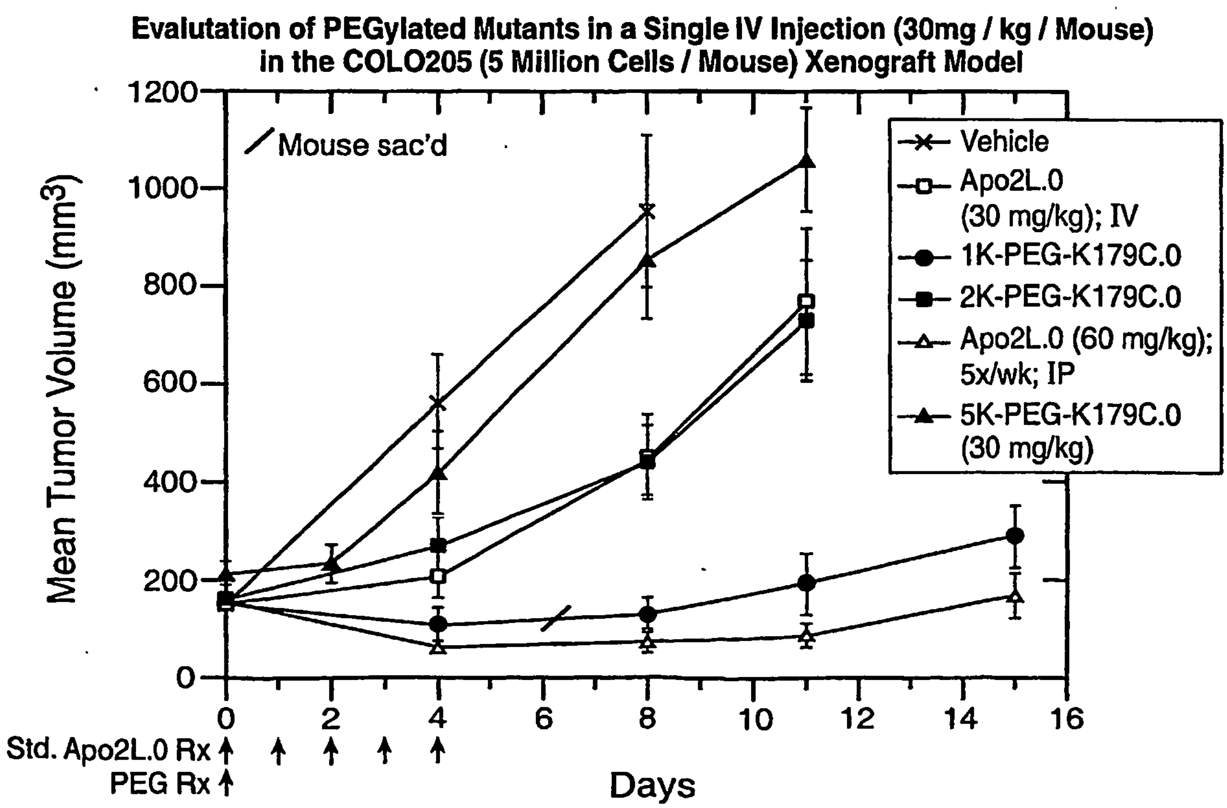

Evalutation of PEGylated Mutants in a Single IV Injection (30mg / kg / Mouse)
in the COLO205 (5 Million Cells / Mouse) Xenograft Model
Mouse sac'd
Vehicle
Apo2L.0 (30 mg/kg); IV
1K-PEG-K179C.0
2K-PEG-K179C.0
Apo2L.0 (60 mg/kg); 5x/wk; IP
5K-PEG-K179C.0 (30 mg/kg)
Mean Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
Std. Apo2L.0 Rx
PEG Rx
Days

FIG._22